A. M. A.
ARCHIVES OF
PATHOLOGY

EDITORIAL BOARD

GRANVILLE A. BENNETT, Chicago, Chief Editor

CHARLES E. DUNLAP, New Orleans
WILEY DAVIS FORBUS, Durham, N. C.
STUART LIPPINCOTT, Seattle
WILLIAM B. WARTMAN, Chicago
GEORGE H. WHIPPLE, Rochester, N. Y.
S. B. WOLBACH, Boston

FEBRUARY 1954
VOLUME 57 NUMBER 2

Published Monthly by
AMERICAN MEDICAL ASSOCIATION
535 NORTH DEARBORN STREET • CHICAGO 10, ILLINOIS

Entered as Second Class Matter Jan. 20, 1926, at the Postoffice at Chicago, Under the Act of March 3, 1879. Annual Subscription, $8.00

TABLE OF CONTENTS FIRST PAGE

PARAGON STAINS **PARAMOUNT QUALITY**

PARAGON STAINING SOLUTIONS

For Tissue Sections

Dependable—Today; Tomorrow; Every Day

With Paragon Staining Solutions you obtain superbly stained tissue sections. The brilliance and sharpness of the stain without diffusion or unpredictable characteristics greatly facilitates diagnosis.

HEMATOXYLIN STAIN—PARAGON (aqueous alum hematoxylin). Made from our own formula. Yields vivid, sharply stained blue nuclei that are really blue—not off color or muddy. Extremely sharp staining and selective with no diffusion. Full bodied and strong. For a given staining time, repeatedly duplicates depth of staining from slide to slide—every day.

PS1101	Bottle (500 cc)	$2.25

EOSIN STAIN—PARAGON (alcoholic). A special eosin compound of our own preparation. Produces deep brilliant red counterstains. Packed in two forms—ready to use and concentrated (requiring the addition of 3 parts of 95% alcohol).

PS1201D	Bottle (500 cc) ready to use	$2.25
PS1201	Bottle (250 cc) for 1000 cc	3.00

ELASTIC FIBER STAIN—PARAGON. Our own resorcin-fuchsin modification of Weigert's Elastic Fiber Stain. Relieves the laboratory of the laborious work involved in the preparation of this important stain. Stains sharply with no diffusion into other tissue components.

PS1225	Bottle (250 cc)	$2.65

VAN GIESON STAIN—PARAGON. Especially designed to produce brilliant differential counterstaining with less tendency to wash out in rinsing alcohols.

PS1250	Bottle (250 cc)	$1.50

PARAGON MULTIPLE STAIN FOR FROZEN SECTIONS. Invaluable to the Pathologist where seconds count and the Surgeon waits for the diagnosis. A single solution which stains instantaneously yielding a hematoxylin-eosin like picture. No special technic. With Paragon Mounting Medium For Frozen Sections (water soluble) section is stained, mounted and under microscope in less than one minute.

PS1301	Paragon Multiple Stain For Frozen Sections	Bottle (50 cc)	$2.00
P451	Paragon Mounting Medium For Frozen Sections	Bottle (25 cc)	.50

Request samples on your institution letterhead.
Write for fully descriptive catalog number 1049 A which includes a descriptive section on staining technics.

All prices F. O. B. New York, New York, subject to change without notice.

Manufactured exclusively by

PARAGON C. & C. CO., Inc. • 2540 Belmont Ave., New York 58, N. Y.

Cable Address: Wijeno, New York.

Write for details on the following Paragon Staining Solutions:

ACID FAST BACTERIA STAIN • CRYSTAL VIOLET STAIN • GRAM'S IODINE SOLUTION
SAFRANIN STAIN • LOEFFLER'S ALKALINE METHYLENE BLUE • ZIEHL-NEELSEN STAIN
WRIGHT'S STAIN • BUFFER SOLUTION FOR WRIGHT'S STAIN

CONTENTS

Original Articles

PAGE

Effects of Age, Growth Hormone, Cortisone, and Other Factors on Collateral Circulation

Murray Z. Rosenberg, M.D., and Averill A. Liebow, M.D., New Haven, Conn. 89

Primary Carcinoma of the Lung in the Male Veteran

K. M. Earle, M.D., Galveston, Texas 106

An Acid-Fast Lipid from Spermatozoa

J. W. Berg, M.D., New York 115

Carcinoma of the Testes Metastatic from Carcinoma of the Prostate

Saul Kay, M.D.; G. R. Henningar, M.D., and J. W. Hooper Jr., M.D., Richmond, Va. 121

Effects of *DL*-Ethionine on the Pancreas and Other Organs

Mario Alvizouri, M.D., México, D. F., Mexico, and Shields Warren, M.D., Boston .. 130

Myelomalacia and Multiple Cavitations of Spinal Cord Secondary to Adhesive Arachnoiditis

Robert L. McLaurin, M.D., Cincinnati; Orville T. Bailey, M.D., Indianapolis; Peter H. Schurr, M.B., London, England, and Franc D. Ingraham, M.D., Boston 138

Varying Content of Nucleic Acids in Polymorphonuclear Leucocytes in Abscesses of Patients

M. L. Menten, M.D., Ph.D., and Mary Willms, M.A., Vancouver, B. C., Canada ... 147

In Vitro Dehydrogenase Activity of Adrenal Slices in Control and Tumor-Bearing Mice

Maurice M. Black, M.D., and Francis D. Speer, M.D., New York 152

Case Reports

Embryological Aneurysm of the Myocardial Vessels

William V. Lovitt Jr., M.D., Baltimore, and Major Stanley Lutz Jr., Medical Corps, United States Air Force 163

Laboratory Methods and Technical Notes

Method for Routine Histopathologic Study of Human Sinoatrial Node

Maurice Lev, M.D., Miami Beach, Fla., and Alvin L. Watne, M.D., Chicago 168

Regular Departments

News and Comment 178

AMERICAN MEDICAL ASSOCIATION

Scientific Publications

The Journal of the American Medical Association. Weekly. Annual Subscription Price, $15.00.

Quarterly Cumulative Index Medicus. Issued Twice a Year. Subscription Price, Calendar year, $20.00.

A. M. A. Specialty Journals

Monthly

A. M. A. Archives of Internal Medicine. Price, $10.00. Paul S. Rhoads, M.D., Chief Editor, American Medical Association, 535 N. Dearborn St., Chicago 10.

A. M. A. Archives of Dermatology and Syphilology. Price, $12.00. Paul A. O'Leary, M.D., Chief Editor, 102 Second Ave. S.W., Rochester, Minn.

A. M. A. Archives of Ophthalmology. Price, $12.00. Francis Heed Adler, M.D., Chief Editor, 313 S. 17th St., Philadelphia 5.

A. M. A. American Journal of Diseases of Children. Price, $12.00. Clifford G. Grulee, M.D., Chief Editor, 610 Church St., Evanston, Ill.

A. M. A. Archives of Pathology. Price, $8.00. Granville A. Bennett, M.D., Chief Editor, Pathology Dept., Univ. of Illinois, 1853 W. Polk St., Chicago 12.

A. M. A. Archives of Neurology and Psychiatry. Price, $12.00. Tracy J. Putnam, M.D., Chief Editor, 450 N. Bedford Drive, Beverly Hills, Calif.

A. M. A. Archives of Otolaryngology. Price, $12.00. George M. Coates, M.D., Chief Editor, 1721 Pine St., Philadelphia 3.

A. M. A. Archives of Surgery. Price, $14.00. Waltman Walters, M.D., Chief Editor, American Medical Association, 535 N. Dearborn St., Chicago 10.

A. M. A. Archives of Industrial Hygiene and Occupational Medicine. Price, $8.00. Prof. Philip Drinker, Chief Editor, Dept. of Industrial Hygiene, Harvard University School of Public Health, 55 Shattuck St., Boston 15.

Prices slightly higher in Canada and Foreign countries. Checks, money orders, and drafts should be made payable to the American Medical Association, 535 North Dearborn Street, Chicago 10.

Instructions to Contributors

Communications regarding editorial management, subscriptions, reprints, etc., should be addressed to Specialty Journals, American Medical Association, 535 North Dearborn Street, Chicago 10.

Articles, book reviews, and other materials for publication should be addressed to the Chief Editor of the Specialty Journal concerned. Articles are accepted for publication on condition that they are contributed solely to that journal.

An original typescript and the first carbon of an article should be provided, and must be double or triple spaced on one side of a standard size page, with at least 1-inch margin at each edge. An article in English by a foreign author should be accompanied by a draft in the author's mother tongue. Improvised abbreviations should be avoided.

The maximum illustration space on a journal page is 5 by 8 inches. Original line drawings should not exceed 8½ by 11 inches. Oversized originals should be photographed and a print submitted within the 5 by 8 limits. Prints larger than 5 by 8 inches will be reduced in scale and/or cropped. Photomicrographs larger than 5 by 8 inches will be reduced in scale unless portions to be cropped are indicated by the author. Any cut-off marks should be made on the margins or mountings rather than on the illustration itself. Charts and drawings should be in black ink on hard, white paper. Lettering must be large enough to permit necessary reduction. Glossy prints of x-rays are requested. Paper clips should not be used on prints, since their mark shows in reproduction, as does writing on the back of prints with hard lead pencil or stiff pen. Labels should be prepared and pasted to the back of each illustration showing its number, the authors name, an abbreviated title of the article, and top plainly indicated. Photographs should not be rolled unless too large to be sent flat. If mailed flat, protect them with several layers of corrugated board. A rolled photograph should face outward. Charts and illustrations must have descriptive legends, grouped on a separate sheet. Tables must have captions.

References to the literature should be numbered in the order in which they are referred to in the text or listed in alphabetical order without numbers. A chronological arrangement, with all entries for a given year alphabetized according to the surname of the first author, may be used if preferred. References should be typed on a special page at end of manuscript. They should conform to the style of the *Quarterly Cumulative Index Medicus*, and must include, in the order given, name of author, title of article (with subtitle), name of periodical, with volume, page, month—day of month if weekly or biweekly—and year. Names of periodicals should be given in full or abbreviated exactly as in the *Quarterly Cumulative Index Medicus*. Reference to books must contain, in the order given, name of author, title of book, city of publication, name of publisher, and year of publication. Titles of foreign articles, if originally in a generally known Romance or Germanic tongue, must either all be in English translation, preferably that used in the *Quarterly Cumulative Index Medicus* subject entries, or all in the original language. Titles in other languages must be translated. The author must assume responsibility for the accuracy of foreign titles.

Matter appearing in the A. M. A. Specialty Journals is covered by copyright, but as a rule no objection will be made to its reproduction in a reputable medical journal if proper credit is given. However, the reproduction for commercial purposes of articles appearing in the A. M. A. Specialty Journals, or in any other publications issued by the Association, will not be permitted.

AMERICAN MEDICAL ASSOCIATION

535 North Dearborn Street **Chicago 10**

How to make a hero out of a half hour . . .

Report accurate prothrombin time determinations at a moment's notice

Simplastin is ready for use in minutes . . . just add distilled water. You can *save* from 30 to 40 precious minutes every time Simplastin is used in prothrombin time determinations. This time saved could mean a life saved for a patient on the critical list receiving anticoagulant therapy.

Simplastin is a time, effort and life saver because costly extra steps demanded in the preparation of ordinary reagents are easily avoided when you standardize with this dependable thromboplastin extract.[1] Avoid the cumulative errors which can occur during extracting, centrifugation or filtering of older preparations.[2]

Simplastin assures your laboratory of more accurate, more reliable reports.[3]

Supplied in 2 sizes

Efficient 6-Determination Size for emergency use and for the smaller laboratory

Economical 20-Determination Size for the larger laboratory

Both sizes are supplied in boxes containing 10 vials. A trial supply will be sent promptly when requested. Available at leading laboratory supply houses. Write for name of your local supplier.

1. Schilling, F. J.; De Natale, A., and Mottram, F. C.: Am. J. M. Sc. *222*:207 (Aug.) 1951. 2. Shapiro, S., and Weiner, M.: J. M. Soc. New Jersey *48*:1 (Jan.) 1951. 3. Shapiro, S., *et al.*: Am. Heart J. *40*:766 (Nov.) 1950.

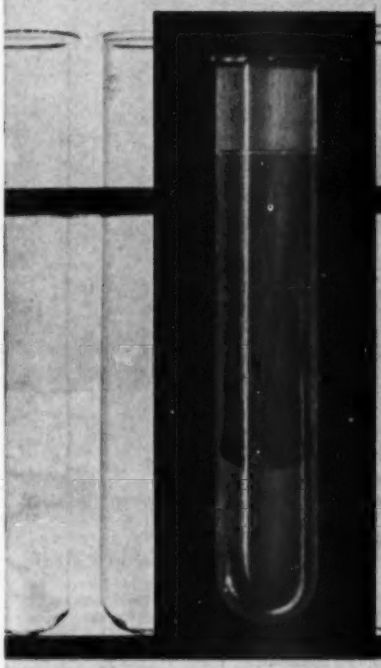
Simplastin®

simple for the technician

accurate for the clinician

WARNER-CHILCOTT *Laboratories*, NEW YORK

REPRODUCIBLE prothrombin determinations



For accurate, consistent, *reproducible* prothrombin time determinations . . . laboratory reports on which the physician can depend without question . . .

SOLU-PLASTIN®

(Thromboplastin Solution-Schieffelin)

A stable, uniform solution, supplied with standardized calcium chloride solution . . . simple and easy to use . . . economical . . . but, most important, *consistent* . . . to produce reproducible results.

SOLU-PLASTIN Supplied in bottles of 10 cc. with like amount of 0.0125M Calcium Chloride Solution

Write for detailed literature.

NEW . . . CRP ANTISERUM: C-reactive Protein Antiserum, Schieffelin . . . for the determination of the acute inflammatory process as an evaluation of therapy in rheumatic fever and other conditions. Literature available on request.

Schieffelin & Co. pharmaceutical and research laboratories since 1794

18 Cooper Square, New York 3, N. Y.

A. M. A.
ARCHIVES OF PATHOLOGY

VOLUME 57 FEBRUARY 1954 NUMBER 2

COPYRIGHT, 1954, BY THE AMERICAN MEDICAL ASSOCIATION

EFFECTS OF AGE, GROWTH HORMONE, CORTISONE, AND OTHER FACTORS ON COLLATERAL CIRCULATION

MURRAY Z. ROSENBERG, M.D.
AND
AVERILL A. LIEBOW, M.D.
NEW HAVEN, CONN.

THE GROWTH and development of collateral arterial channels following the ligation or occlusion of a major artery is a well-recognized but poorly understood phenomenon. It is apparent from the study of such organs as the lung under appropriate circumstances that some collateral vessels represent merely expanded preexisting channels but that others are derived from the growth of capillaries in newly formed connective tissue. It may well be that there are diverse mechanisms controlling these separate sources.

That the primary stimulus may be mechanical was suggested by Holman,[1] who countered Lewis'[2] suggestion that metabolic products of ischemic tissue may open up collateral channels by showing that collateral circulation will develop about an arteriovenous fistula despite amputation of ischemic tissue. It was Holman's contention that collateral channels expand after ligation of a large artery in response to a transformation of high "end pressure" in the ligated vessel to "lateral pressure." That the pressure in lateral branches rises proximal to obstruction of a main arterial trunk was demonstrated by experiment. The pressure increment was presumed to force an additional volume of blood, formerly carried by the obstructed main channel, through collateral branches. By mechanisms not entirely clear, vessels with an increased blood flow tend to enlarge. Mulvihill and Harvey[3] gave early recognition to the role of the sympathetic nervous system in adjustments of blood flow following arterial ligation and demonstrated a relatively rapid return to normal temperature in an extremity to which the major artery of supply was ligated and a maintenance of normal temperature if sympathectomy was performed with the ligation. Reflex mechanisms initiated by increasing the temperature of distant regions of the body have also been described and applied in therapy (Learmonth[4]).

That the formation and development of new vascular channels may be modified or controlled by factors other than purely mechanical or reflex adjustments is suggested by recent studies of the effects of cortisone and corticotropin (ACTH) on mesenchymal tissue subjected to various types of injury. The vascularization of the cornea which occurs after exposure to irritant chemicals, such as sodium hydroxide, has been shown to be inhibited by corticotropin.[5] Several investigators have demonstrated a pronounced inhibition of new vessel formation in granulation tissue by

From the Department of Pathology, Yale University School of Medicine; supported by a grant from the Office of Naval Research under contract number N6ori-44, Task Order XI.

* References 6 to 10.

cortisone and corticotropin.* Diminution in fibroplasia has been consistently recognized, along with the failure in the development of the vascular component of granulation tissue, and it has not been clear in just what way these were related. Failure in formation of granulation tissue has been offered in explanation of faulty wound healing observed in mice,[11] rats,[12] rabbits,† and man [18] treated with cortisone and corticotropin. Taubenhaus and Amromin [6] have implicated other hormones as affecting the formation of granulation tissue. These investigators studied turpentine-induced abscesses in rats and concluded that cortisone, hypophysectomy, and sex hormones inhibit granulation tissue formation, whereas growth hormone and desoxycorticosterone acetate are stimulating.

The suggestion that hormones might influence the development of collateral channels following ligation came from the observation that in newborn puppies in which a pulmonary artery was ligated the growth of bronchial arterial collaterals was remarkably greater than the growth of these vessels over a similar interval of time in adult dogs subjected to the same operation.[19] We have been unable to find reports of any other studies designed to determine what role age and hormonal factors may play in the development of collateral circulation. The present study was undertaken in an attempt to elucidate the effects of age and pituitary and adrenal hormones on the development of new arterial channels following ligation of a major artery.

METHODS

Although the observations leading to the present experiments had been made on young dogs, the rat was chosen as the experimental animal so that a large series might be employed to minimize the error produced by uncontrollable differences among individuals. The right common iliac artery was chosen for ligation after it was found that interruption of the lower abdominal aorta was accompanied by a formidable mortality. This vessel was considered well suited to the purposes of the experiment, being one of a pair of symmetrical arteries and thus allowing comparison with the arteries of the opposite side. Rats were divided into six groups as follows:

Group A—Adult male rats, approximately 350 gm., Sprague Dawley Strain,‡ as controls

Group B—Rats§ of same sex, weight, strain, and age as Group A given daily injections of 0.4 mg. (approximately 1 mg. per kilogram) of growth hormone preparation J-21609R,‖ beginning on day of operation

Group C—Rats¶ of same sex, weight, strain, and age as Group A given daily injections of 5 mg. of cortisone (Cortone#) acetate, beginning two days prior to operation

Group D—Immature male rats,* about 150 gm., Sprague Dawley Strain

Group E—Hypophysectomized male rats,† 200 gm., Sprague Dawley Strain

Group E_{cr}—Controls for Group E; normal rats of same strain and weight as hypophysectomized rats at the time of ligation but somewhat younger

The right common iliac artery was doubly ligated and severed at its origin in all rats. The anesthetic was pentobarbital (Nembutal), given intraperitoneally, 4 mg./100 gm. of body weight, in all rats except those of Group E, which showed a marked reduction in tolerance to

† References 13 to 17.

‡ Rats obtained from Sprague Dawley Co., Madison, Wis.

§ Rats obtained from Sprague Dawley Co., Madison, Wis.

‖ This preparation was supplied by Armour and Company and is approximately equivalent in potency to Wilhelmi's purified growth hormone preparation.

¶ Rats obtained from Sprague Dawley Co., Madison, Wis.

Merck & Company, Inc.

* Rats obtained from Sprague Dawley Co., Madison, Wis.

† Rats obtained from Hormone Assay Laboratories, Inc., Chicago.

anesthesia and were maintained with 2 mg./100 gm. of body weight and additional 0.25 mg. doses as needed. A midrectus incision was used, and the approach was transperitoneal. Closure was effected with a single layer of silk sutures to fascia and peritoneum and clips to the skin.

Rats were weighed periodically and observed for differences in wound healing, hair growth, and changes in the right lower extremity. All surviving rats were then killed at 15 weeks postoperatively with an overdose of pentobarbital. Intracardiac heparin sodium was administered terminally to delay intravascular clotting. Organs weighed at death included thyroid, thymus, heart, spleen, and testes. Sections for microscopic study were obtained from pituitary, thyroid, thymus, heart, liver, spleen, adrenal, kidney, and both skin and muscle wounds.

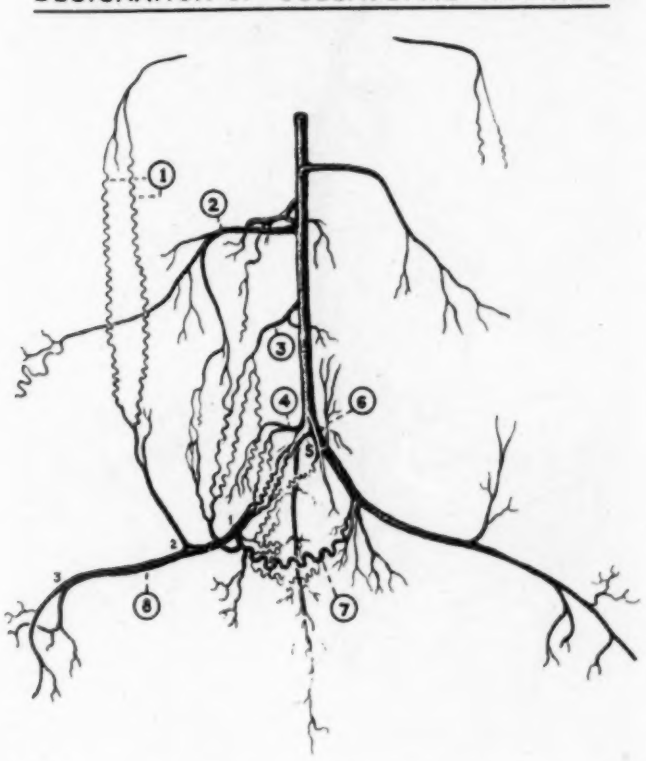


Fig. 1.—For purposes of providing an objective evaluation of the development of collateral circulation, certain key points of reference were established, as indicated in the diagram by the following numbers: *1*, right intercostal vessels and inferior epigastric; *2*, right iliolumbar vessels; *3*, right lateral branches of dorsal vessels; *4*, right lateral branches of caudal vessels; *5*, expanded collaterals from right common iliac; *6*, expanded collaterals from inferior mesenteric; *7*, vessels crossing midline from left hypogastric; *8*, old distal vessel to ligated right common iliac. The sketch corresponds to the actual Vinylite cast shown in Figure 2.

The descending thoracic aorta was cannulated and perfused with 10 cc. of isotonic saline. Postmortem angiography was then performed by injecting 1.5 cc. of colloidal thorium dioxide (Thorotrast) into the cannulated aorta over a two-second interval, with exposure at the end of the injection.

After the angiography, corrosion casts were made of the arterial tree below the diaphragm, using 9% Vinylite‡ in acetone under pressure maintained for a period of 24 hours by a rubber band of 3-in. (7.62 cm.) circumference wrapped three times about the barrel of the syringe and looped over the plunger. The plunger end of the syringe was "capped" with the finger of a rubber glove containing acetone to prevent "freezing" at the interval. After three to four days of digestion in concentrated HCl, casts were gently washed with water for 24 hours, dried, and

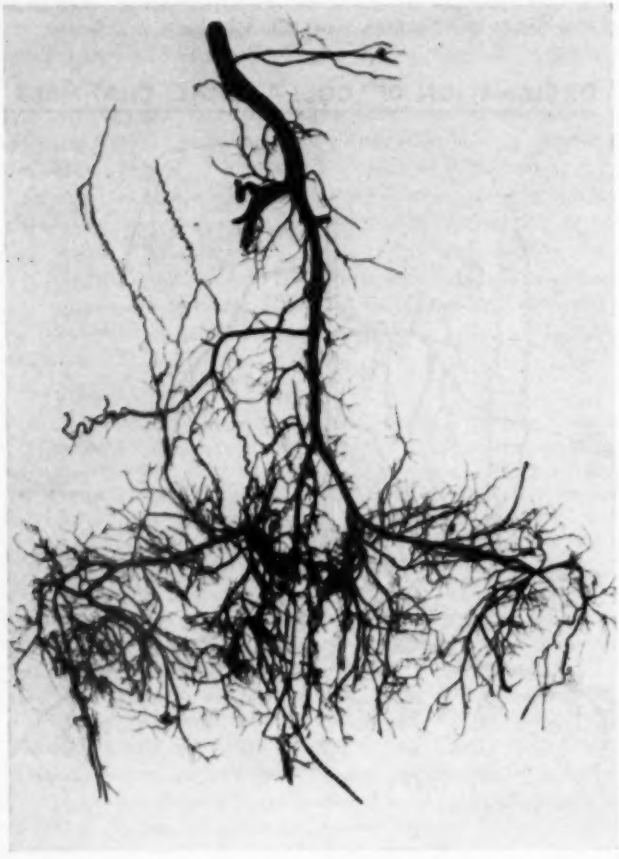


Fig. 2 (Rat A3 [Control]).—Vinylite cast of aorta and its branches, including the collaterals through which the distal segment of the right iliac artery was filled. Compare with Figure 1.

washed with petroleum benzin to remove residual fat. Every effort was made to standardize the procedure and to minimize trauma to the delicate casts.

The goal of achieving objectivity in evaluating the extent of the collateral circulation was paramount. To this end several of the more important collateral channels were identified and designated, as indicated in Figures 1, 2, and 3. These could be identified both in the roentgeno-

‡ VYHH, Specification No. 1, made by Union Carbon and Carbide Chemicals Corp., Bakelite Division, 437 MacCorkle Ave., South Charleston, W. Va.

grams and in the casts. In estimating the degree of expansion of the collateral circulation, the maximum demonstrable in either the casts or roentgenograms was scored. Collateral channels numbered 1 to 7 were scored as follows:

0—No Expansion: For Channels 1 to 4, same as contralateral side; for Channels 5 and 6, no evident new vessel formation; for Channel 7, no vessels crossing midline, line of aorta being used as midline

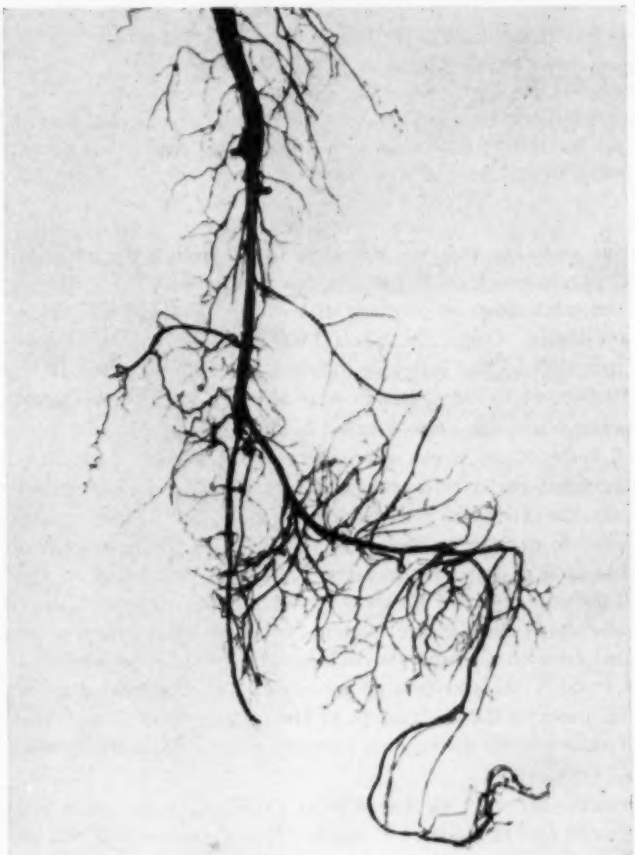


Fig. 3 (Rat C3).—Rat was cortisonized for 15 weeks. There is expansion of collateral vessels from the right iliolumbar, dorsal, and caudal groups (Channels 2, 3, and 4) and to a slight extent from the right common iliac, but the distal iliac segment has not been injected on the right. On the left the plastic has penetrated into the arteries of the foot.

1—Beginning Expansion of Channels: For Channels 1 to 4, larger caliber, more branches, or greater length than contralateral side; for Channels 5 and 6, new vessels evident; for Channel 7, vessels crossing midline; for all channels, no communication with distal ligated vessel

2—Communicating Channels of Increased Size: Same as 1 for all channels, except that communication with distal ligated vessel is evident; total caliber of new vessels or vessels not visible on contralateral (unoperated) side is less than that of left iliolumbar artery

3—Communicating Channels of Large Size: Same as 2 for all channels, except that total caliber of new vessels or vessels not visible on opposite side is approximately equal to or greater than that of the left iliolumbar artery

Channel 8, the artery distal to the ligature, provided some indication of total collateral and was scored as follows:

0—No filling with plastic and filling with colloidal thorium dioxide to less than one-half the contralateral vessel in length

1—Filling to origin of right hypogastric artery with plastic or filling with colloidal thorium dioxide greater than one-half of the contralateral (control) artery

2—Filling to origin of right inferior epigastric with plastic

3—Filling to end of right femoral artery with plastic

4—Filling to vessels of right foot with plastic or filling greater on right than left

To eliminate bias, rats of different groups were mixed and given code numbers. Scoring was performed independently by each of three observers.

OBSERVATIONS

Operative mortality was less than 5% in all groups, except group E (hypophysectomized), in which death within three days approached 90%, despite the fact that with the small doses of pentobarbital employed almost all rats in this group survived anesthesia. Originally, adult (400 gm.) hypophysectomized rats were used; the mortality in this group approached 100%. Only 2 of 32 hypophysectomized rats subjected to the operation were alive 15 weeks after operation.

The operative wounds were observed in all groups periodically and were superficially well healed three weeks after operation. By about the fifth postoperative week it was noted that cortisone-treated rats were developing incisional hernias. Of the six rats maintained on cortisone over the 15-week interval of the experiment, only one failed to develop an incisional hernia. These hernias gradually increased in size and in some cases contained about 50% of the volume of the contents of the abdomen at the time of death. Of the 47 rats of other groups surviving the 15-week interval, none showed evidence of wound herniation at the time of death. A cortisone-treated rat with a representative large hernial sac exposed at death is shown in Figures 4 and 5. This evident propensity of cortisone-treated rats to incisional hernias is in line with the observation of Howes and co-workers [20] that the tensile strength of skin wounds undergoing primary closure is markedly reduced in rats treated with cortisone.

Hair growth over the area shaved prior to operation was scant to absent in all cortisone-treated rats at the time of death. Hypophysectomized rats showed somewhat less hair growth than controls, but the difference was not nearly as striking as in the cortisone-treated group. All rats of other groups showed abundant hair growth, and originally shaved areas were not identifiable externally eight weeks after operation. A cortisone-treated rat showing the poor hair growth noted in all six rats of this group is shown in Figure 4 in comparison with a control (Fig. 6). Corticotropin inhibition of hair growth has been noted in animals by others.§ Corticotropin is reported by Behrman and Goodman [24] to produce hypertrichosis in man.

The factors of initial weight as a measure of size or of change in weight after ligation were considered as possibly important determinants in the development of

§ References 21 to 23.

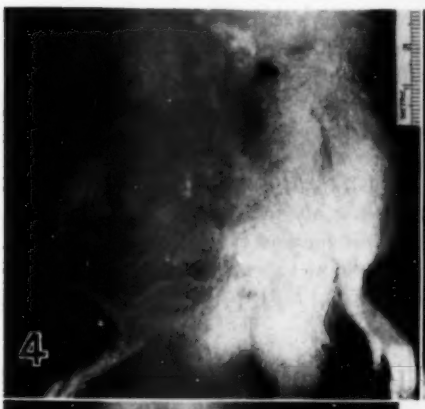


Fig. 4 (Rat C6).—Rat was cortisonized for 15 weeks. Large hernia protruding from the region of incision. Hair growth is scanty over the lower abdomen and over the hernia sac in the operative site.

Fig. 5 (Rat C6).—Rat was cortisonized for 15 weeks. Hernia sac exposed showing exceedingly thin skin and defect in muscular wall.

Fig. 6 (Rat A1 [Control]).—Well-healed scar and intact abdominal wall. Hair growth is abundant in the abdominal region shaved prior to operation.

collateral circulation. The weights of the animals in various groups are recorded in Table 1. Control rats (Group A) maintained fairly stable average weights, as follows: initial, 349 gm.; 5 weeks, 359 gm.; 11 weeks, 375 gm.; 15 weeks, (at death), 358 gm. Rats treated with growth hormone (Group B) showed a slight gain in mean body weights from an average of 355 gm. at the time of ligation to an average of 373 gm. at necropsy. This gain lacked statistical significance.‖ The animals that received cortisone (Group C) lost weight at first but then regained a little, as follows: initial, 350 ± gm.; 3 weeks, 275 gm.; 9 weeks, 280 gm.; 15 weeks (at death), 311 gm. At the final weighing there was still a statistically significant difference between these animals and the controls ($P < 0.02$). Young rats (Group D) gained steadily in weight and reached adult size after 15 weeks, nearly tripling their weight at the time of operation. Hypophysectomized rats (Group E) failed to gain weight during the 15-week interval, and the mean weight of this group was less than one-half that of their control group (Group E_{c2}) at the time of death.

TABLE 1.—*Weights of Control and Experimental Groups of Rats*

Group	Wt. at Ligation, Gm.	Wt. at Death (15 Weeks), Gm.
A (controls for B, C, D)	Av. 349 Range 300-395	Av. 358 (12 rats) Individual: 305, 305, 343, 350, 353, 353, 367, 377, 380, 383, 387, 393
B (growth hormone)	Av. 355 Range 335-385	Av. 373 (10 rats) Individual: 302, 343, 355, 365, 372, 378, 384, 399, 410, 420
C (cortisone)	Av. Presumed same as A	Av. 311 (6 rats) Individual: 283, 296, 308, 308, 310, 360
D (young rats)	Av. 128 Range 120-145	Av. 372 (10 rats) Individual: 305, 338, 352, 356, 365, 367, 384, 385, 428, 436
E (hypophysectomized young rats)	Av. 200±	Av. 144 (2 rats) Individual: 143.5, 144
E_{c1} (controls for old hypophysectomized rats)	Av. 400±	Av. 413 (6 rats) Individual: 375, 395, 397, 425, 430, 455
E_{c2} (controls for E)	Av. 200±	Av. 339 (7 rats) Individual: 298, 315, 333, 352, 355, 356, 366

The loss of body weight in the cortisone-treated rats is not surprising in view of the conclusive evidence of the protein catabolic or "antianabolic" and growth-inhibiting effects of corticotropin and cortisone (reviewed by Sprague [25]). The gradual regain of lost weight after the third week suggests that some adaptation to the large doses of cortisone may have taken place. Ingle and co-workers,[26] studying nitrogen loss in rats given cortisone, observed that the peak of nitrogen loss was not sustained during continued administration of the hormone and suggested that some adaptation to the catabolic effects of corticotropin and cortisone takes place. Wolfson and co-workers,[27] noting that some diseases become refractory to continued administration of cortisone and corticotropin, suggested that the failure of response may be due to depression of thyroid function. Indeed, Hardy and co-workers [28] observed a decreased serum-protein-bound iodine in two patients after treatment with cortisone and in several after treatment with corticotropin.

The condition of the right lower extremity was noted ante mortem in all rats. During the first 24 hours postoperatively some weakness, coldness, and cyanosis were noted in some rats of all groups. Thereafter no gangrene, paralysis, or other

‖ The "t" test was employed in this and all subsequent statistical analyses.

evident differences on comparison with the contralateral extremity were observed, except in the hypophysectomized group. These rats, most of which died within three days of operation, had gnawed and bleeding right feet, even when segregated, and one of the two rats surviving the 15-week interval showed loss of nails and terminal digits of the right foot.

The results of the scoring of collateral channels in five of the original seven groups are recorded in Table 2. The independent scores of all three observers are indicated. Since none of the old hypophysectomized rats (Group E) lived to the end of the 15-week experimental period, there is no statistically significant data for this group, and their controls (Group E_{c2}) are also omitted. Both of these hypo-

TABLE 2.—*"Collateral Equivalents" of Various Groups of Rats After Ligation of Right Common Iliac Artery**

Group	Av. Equivalent			Individual Equivalents †
	Obs. 1	Obs. 2	Obs. 3	Obs. 2
A (controls for B, C, D)	11.8 ± 0.81	14.5 ± 1.08	11.3 ± 0.66	9, 10, 11, 12, 13, 14, 16, 16, 17, 18, 19, 19
B (growth hormone)	12.6	15.5 ± 0.76	11.7	10, 13, 15, 15, 16, 17, 17, 17, 17, 18
C (cortisone)	8.1 ± 1.81	10.3 ± 1.53	10.0 ± 2.08	6, 8, 8, 9, 14, 17
D (young rats)	11.0	13.1	11.0	7, 9, 12, 13, 14, 15, 15, 15, 15, 16
E_{c1} ("old" controls)	13.6	16.1	13.9	14, 16, 16, 16, 17, 18

* Obs., observer.
† Each equivalent represents the sum of the values for the various channels shown in Figures 1 and 2, scored as indicated under "Methods." In Groups A, B, and C the standard errors of the means are recorded where calculated.

TABLE 3.—*Method of Scoring Collateral*

	Observer		
	1	2	3
By casts alone	56%	63%	47%
By x-rays alone	14%	20%	16%
By casts and x-rays	30%	17%	37%

physectomized animals did show a moderately well-developed collateral circulation. Data concerning Group E_{c1}, comprised of old (400 gm.) rats originally intended as controls for the mature hypophysectomized animals, are of special interest for comparison with other groups and have therefore been tabulated. The relative extents to which casts or x-rays were employed in scoring by the three observers are indicated in Table 3. It is evident that, although most ratings were made on the basis of the casts alone, roentgenograms provided valuable supplementary evidence, especially as a control over possible fragmentation of the casts in handling.

Inspection of Table 2 shows that all three observers scored the various groups in the same relative order of magnitude, although the absolute values differ somewhat. This speaks well for the technique of scoring. It will be recalled that prejudice was avoided by identifying each specimen merely by a code number and by intermingling representatives of the various groups at random.

The scores of all three observers indicated that the animals that had received cortisone (Group C) had developed the least evidence of collateral circulation.

Statistical analysis applied to the results of observer No. 2 showed the probability of error to be < 0.05, a value generally considered to be significant.

The animals that had received growth hormone (Group B) stood somewhat higher than their controls, although the results lack statistical significance. The young animals whose weight had increased by a factor of more than 2.5 stood slightly below the controls in total collateral circulation. The most surprising observation was that the old rats had developed the best collateral circulation, as judged by the criteria employed. This did not meet the test of statistical significance, although the data of all three observers were again in agreement.

The fact that the old control rats (E_{c1}) appeared to develop at least as extensive collaterals as the young growing rats (D) and perhaps more suggests that the initial size of potential collateral channels may be a determinant of their ultimate size. That the ultimate size of an animal also may influence the magnitude of a collateral bed

TABLE 4.—*Mean Weights of Organs of Various Groups of Rats**

		Wt. of Organs (Means)									
		Spleen		Thymus		Heart		Thyroid		Testis	
Group	No. of Rats	Absolute, Mg.	Mg./100 Gm.	Absolute, Mg.	Mg./100 Gm.	Absolute, Mg.	Mg./100 Gm.	Absolute, Mg.	Mg./100 Gm.	Absolute, Mg.	Mg./100 Gm.
A (controls for B, C, D)	12	643	179	333	93	1,180	329	30	8	3,380	941
B (growth hormone)	10	708	214	376	108	1,261	339	34	9	3,400	947
C (cortisone)	6	1,147	308	369	125	1,251	402	33	9	3,180	1,022
D (young rats)	10	840	226	431	116	1,217	327	39	10	3,280	881
E (hypophysectomized)	2	408	283	174	120	662	459	28	19	252	175
E_{c1} (old rats)	10	699	160	464	112	1,309	317	47	11	3,520	851
E_{c2} (controls for E)	7	1,402	413	441	130	1,256	382	29	6	3,400	1,002

* Weights of animals are given in Table 1.

is suggested by the difference, slight as it appears to be, between those animals receiving growth hormone and their controls. In Group D, the young rats, the development of the circulation appeared to be commensurate with the final weights of animals, as judged by the controls. This is of interest in that the considerable somatic growth during the interval of the development of the collaterals appeared to have no special influence, contrary to what might have been expected from the observations on the pulmonary circulation in puppies mentioned before ¶; the collateral circulation in the puppies, however, was induced within 48 hours after birth. In the same sense, it is possible that the small size of the animals in the cortisonized group at the end of the experiment accounts for the small absolute size of the collateral bed. The important conclusion is that cortisone over a 15-week interval does not abolish the capacity for development of collateral circulation. The animals that seemed to have suffered the greatest circulatory disturbances were the hypophysectomized, as suggested by the condition of the lower extremities on the side of the ligature, as mentioned above. No changes of this sort were observed in the cortisonized animals.

¶ References 19 and 29.

Since relatively little information is available on animals kept on high doses of cortisone for as long as 15 weeks in comparison with controls and animals subjected to other forms of hormonal imbalance, the weights of certain organs are presented in Table 4. Histological sections were made of tissues from two to four animals, usually the latter number in each group, and, while they cannot be discussed here in detail, certain relevant features will be mentioned. The most striking observation, in view of what might be expected earlier in the course of treatment with cortisone,# is the high weight, absolute as well as per 100 gm. of body weight, of the spleen

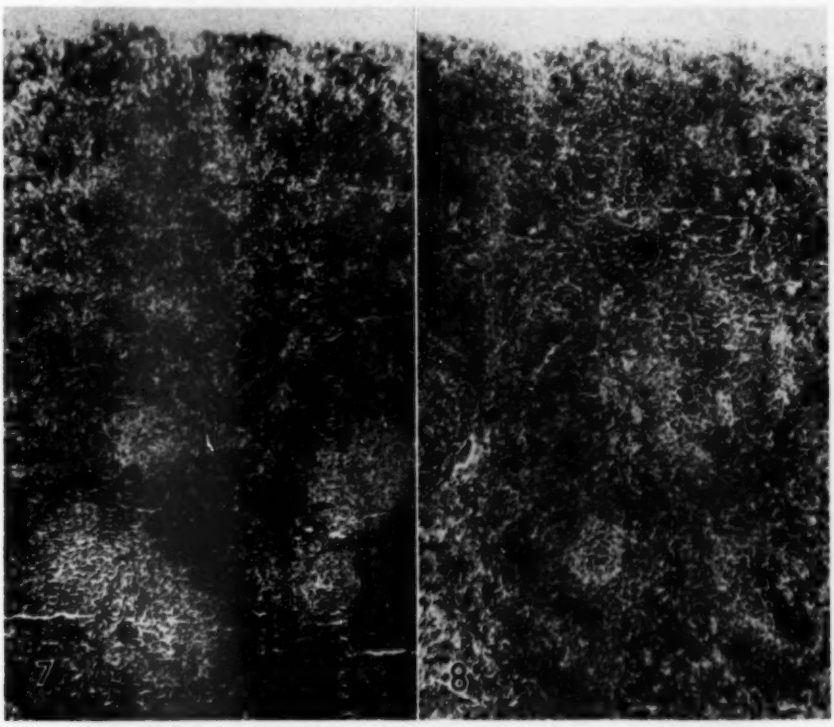


Fig. 7 (Rat C4).—Rat was cortisonized for 15 weeks. Spleen. Germinal centers generally small and few, with exceptions (lower portions of field). Scanty large mononuclear cells about follicles. Much of the bulk of the organ consists of erythrocytes, which appear dark in the photograph. Compare with Figure 8.

Fig. 8 (Rat A7 [Control]).—Numerous and relatively large follicles. Extensive halo of large mononuclear elements about each follicle.

and apparently of the thymus in this group (compare Groups C and A). Microscopically the spleens of three animals in the cortisone group had more scattered and smaller lymphoid follicles with not so large a crown of large pale mononuclear cells about them as did the spleens of the animals of any other group, i. e., the spleen consisted relatively more of blood (Figs. 7 and 8). The spleens of the old

#References 9 and 31.

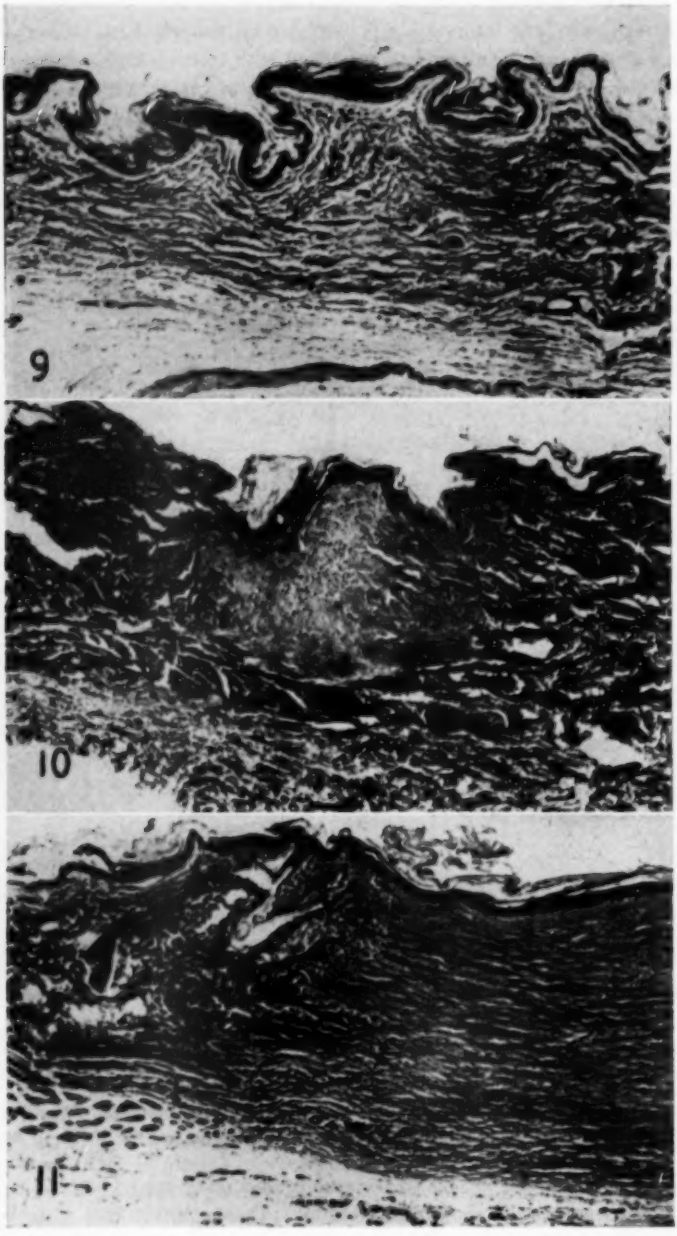


Fig. 9 (Rat C3).—Rat was cortisonized for 15 weeks. Areolar arrangement of connective tissue in region of operative scar. Cutaneous appendages are absent.

Fig. 10 (Rat C2).—Rat was cortisonized for 15 weeks. Small dense scar contrasting with similarly treated animal (Fig. 9).

Fig. 11 (Rat A11 [Control]).—Dense scar tissue. Hair follicles at margins.

rats (Group E_{e1}) were atrophic, especially when considered in relation to body weight. In the case of the thymus, although the weights were apparently large, microscopic studies show that in three of four specimens examined the tissue consisted largely of fat. In these three instances the true thymic substance was in an advanced stage of involution, although some lymphocytes were still present. The lymphoid tissue of small nodes that had been included was also strikingly atrophic in two of the three specimens where there was involution of the thymus. In one

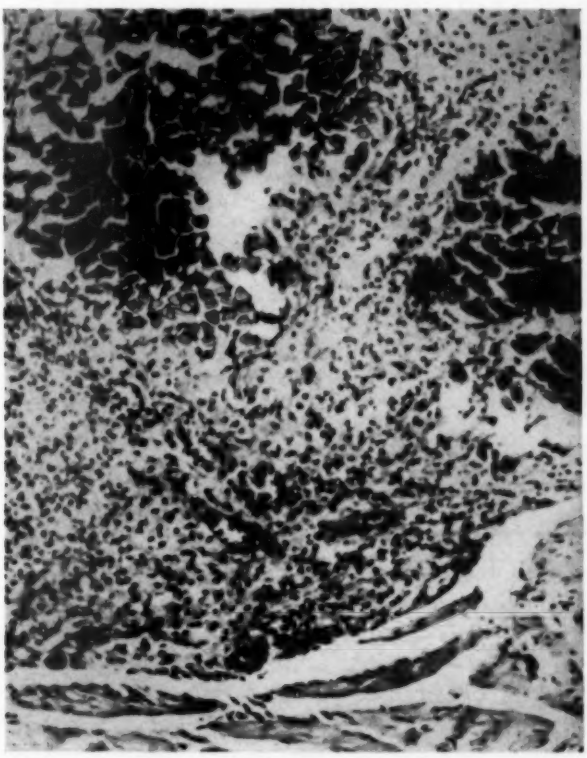


Fig. 12 (Rat C3).—Rat was cortisonized for 15 weeks. Reaction to silk suture material. Relatively abundant granulation tissue, in contrast with Figure 13.

specimen thymic tissue was present in abundance, with well-differentiated cortical and medullary layers.

It is of interest to note that the absolute and even more so the relative weight of the heart was high in the cortisone group as compared with control Group A. The testes and thyroid in the animals on cortisone dosage showed no histological evidence of atrophy, and their weights were close to those of the controls. As was to be expected, these two organs appeared atrophic histologically in the hypophysectomized animals. The adrenals of the cortisonized and hypophysectomized animals were also atrophic. Their weights are not recorded, since these organs were

not removed until the Vinylite injections of the aorta had been completed; this was necessary in order to avoid possible damage to vascular channels in the dorsal region, including possible collaterals.

Groups D and E_{c2} should be roughly comparable, and they appear to be so, except for the strikingly high weights of the spleens in the latter group. This discrepancy is unexplained.

A study of the skin and tissues of the body wall from the cortisone group is of interest (Figs. 9, 10, and 11). An example is the material from rat C_3 (Fig. 9).

Fig. 13 (Rat C2).—Rat was cortisonized for 15 weeks. Reaction to silk suture material consists chiefly of large mononuclear cells and mast cells. Granulation tissue is scanty, and the collagenous capsule is thin.

In this animal the region of the wound and hernia is remarkable for the loose arrangement of its well-defined collagen bundles, that are not far different from the subcutaneous tissue at a distance from the actual incision. There is a very wide gap between the rectus muscles of the abdominal wall, and the one of these seen in the margin of the section has become thinned into a narrow flat band. In the deeper tissues of the body wall, fibroblasts and capillaries form a thin but well-defined layer of granulation tissue about remnants of silk sutures (Fig. 12). In the two other available specimens, C_2 and C_4, numerous large cells with fine baso-

philic granules, considered to be mast cells from their appearance in the hematoxylin and eosin preparations, are seen among the collagen bundles, especially in and near the line of incision but even at a distance from it. In C_2 (Fig. 13) mast cells predominate about residual suture material, and granulation tissue is very scanty. The essential difference of the healing process from that of controls, as seen in its end stages, then appears to lie in the less extensive deposition of dense scar tissue in the cortisonized animals, as is to be expected from the observations of others. The hair follicles in one animal of Group C did not differ qualitatively from the controls. In two others, however, they were rather widely scattered, small, although mature, and the sebaceous glands were almost all in a state of atrophy.

COMMENT

While it is apparent that cortisone in moderate doses administered over a long interval of time does not prevent some expansion of preexisting collaterals, these observations yield no information as to a possible inhibiting effect in the development of new collaterals, such as might come from capillary vessels in newly formed adhesions or in other types of granulation tissue. Even the expansion in size of preexisting vessels, however, doubtless involves the proliferation of elements of connective tissue, among them muscle fibers, as suggested by observations on the lung.[29] It may be that there has been "escape" from an earlier inhibiting action, such as was noted in regard to the weights of the animals in Group C of the present experiment and as suggested also by the high absolute weights of certain organs at the end of the 15-week period of observation. An "escape" phenomenon has been described for other aspects of cortisone activity. Thus incised wounds or fractures produced in animals under cortisone dosage will ultimately heal,[30] and hair follicles, originally inhibited, apparently reach a refractory state after six months of local application of cortisone to the skin.[28]

The question of adequacy of dosage may be raised, especially in consideration of Ragan's [30] statement regarding the relative insensitivity in certain respects of the rat to this material. Shapiro, Taylor, and Taubenhaus,[10] however, demonstrated typical early effects on granulation tissue in rats receiving doses of 5 mg. per day. Furthermore, several findings in the present experiment suggest the adequacy of dosage. These are the loss of weight, a profound effect on connective tissues involved in healing of an abdominal incision as shown by the development of massive hernias, and an atrophy of lymphoid tissue and of the adrenals, persisting in some animals to the end of the 15th week.

SUMMARY AND CONCLUSIONS

By the end of 15 weeks of continuous administration, cortisone only partially inhibits the expansion of the presumably preexisting collateral circulation after ligation of the common iliac artery in the rat; this does not imply that this material may not more profoundly influence the development of new collaterals from granulation tissue. Whether this is a direct effect on the vessels or mediated through other factors, such as those responsible for loss of weight, has not been determined. It would appear that the absence of the hypophysis is associated with more serious immediate functional impairment of the circulation to the extremities after ligation of the common iliac artery than the administration of cortisone.

The initial size and perhaps also the final size of an animal are important determinants of the ultimate magnitude of an induced expansion of a presumably preexisting collateral bed.

Within the limits of the age of subjects and the duration of the present experiment mere rapid somatic growth does not necessarily imply a growth of collateral channels in excess of what might be expected from the final weight.

Hernias usually appear in the region of abdominal incisions of rats maintained on a dosage of 5 mg. of cortisone acetate per day, and in such animals curtailed hair growth and atrophy of adrenal and lymphoid tissues usually persist for at least 15 weeks.

REFERENCES

1. Holman, E.: Problems in the Dynamics of Blood Flow: Conditions Controlling Collateral Circulation in the Presence of an Arteriovenous Fistula, Following the Ligation of an Artery, Surgery **26**:889, 1949.

2. Lewis, T.: Adjustment of Bloodflow to Affected Limb in Arteriovenous Fistula, Clin. Sc. **4**:277, 1940.

3. Mulvihill, D. A., and Harvey, S. C.: The Mechanism of the Development of Collateral Circulation, New England J. Med. **204**:1032, 1931.

4. Learmonth, J.: Collateral Circulation, Natural and Artificial, Surg., Gynec. & Obst. **90**:385, 1950.

5. Jones, I. S., and Meyer, K.: Inhibition of Vascularization of Rabbit Cornea by Local Application of Cortisone, Proc. Soc. Exper. Biol. & Med. **74**:102, 1950.

6. Taubenhaus, M., and Amromin, G. D.: The Effects of the Hypophysis, Thyroid, Sex Steroids, and the Adrenal Cortex upon Granulation Tissue, J. Lab. & Clin. Med. **36**:7, 1950.

7. Ducommun, P., and Mach, R. S.: L'action de l'A.C.T.H. sur les adhérences dues à l'injection de talc dans la cavité péritonéale des rats: Communication preliminaire, Semaine hôp. Paris **26**:3170, 1950.

8. Selye, H.: Further Studies Concerning Participation of Adrenal Cortex in Pathogenesis of Arthritis, Brit. M. J. **2**:1129, 1949.

9. Spain, D. M.; Molomut, N., and Haber, A.: The Effect of Cortisone on the Formation of Granulation Tissue in Mice, Am. J. Path. **26**:710, 1950.

10. Shapiro, R.; Taylor, B., and Taubenhaus, M.: Local Effects of Cortisone on Granulation Tissue and the Role of Denervation and Ischemia, Proc. Soc. Exper. Biol. & Med. **76**:854, 1951.

11. Spain, D.; Molomut, N., and Haber, A.: Biological Studies on Cortisone in Mice, Science **112**:335, 1950.

12. Baker, B. L., and Whitaker, W. L.: Interference with Wound Healing by the Local Action of Adrenocortical Steroids, Endocrinology **46**:544, 1950.

13. Ragan, C.; Howes, E. L.; Plotz, C. M.; Meyer, K.; Blunt, J. W., and Lattes, R.: The Effect of ACTH and Cortisone on Connective Tissue, Bull. New York Acad. Med. **26**:251, 1950.

14. Plotz, C. M.; Howes, E. L.; Meyer, K.; Blunt, J. W.; Lattes, R., and Ragan, C.: The Effect of the Hyperadrenal State on Connective Tissue, Am. J. Path. **26**:709, 1950.

15. Blunt, J. W.; Plotz, C. M.; Lattes, R.; Howes, E. L.; Meyer, K., and Ragan, C.: Effect of Cortisone on Experimental Fractures in the Rabbit, Proc. Soc. Exper. Biol. & Med. **73**:678, 1950.

16. Cavallero, C.; Borasi, M.; Sala, G., and Amira, A.: Effetto del cortisone, del DCA e dell'artisone sulla guarigione dell ferite cutanee sperimentali, Arch. internat. pharmacodyn. **86**:43, 1951.

17. Baxter, H.; Schiller, C., and Whiteside, J. H.: The Influence of ACTH on Wound Healing in Man, Plast. & Reconstruct. Surg. **7**:85, 1951.

18. Creditor, M. C.; Bevans, M.; Mundy, W. L., and Ragan, C.: Effect of ACTH on Wound Healing in Humans, Proc. Soc. Exper. Biol. & Med. **74**:245, 1950.

19. Liebow, A. A.; Harrison, W., and Hales, M. R.: Experimental Pulmonic Stenosis, Bull. Internat. A. M. Mus. **31**:1, 1950.

20. Howes, E. L.; Plotz, C. M.; Blunt, J. W., Jr., and Ragan, C.: The Retardation of Wound Healing by Cortisone, Surgery **28**:177, 1950.

21. Asling, C. W.; Reinhardt, W. O., and Li, C. H.: Effects of Adrenocorticotropic Hormone on Body Growth, Visceral Proportions, and White Blood Cell Counts of Normal and Hypophysectomized Male Rats, Endocrinology **48**:534, 1951.

22. Moon, H. D.: Inhibition of Somatic Growth in Castrate Rats with Pituitary Extracts, Proc. Soc. Exper. Biol. & Med. **37**:34, 1937.

23. Castor, C. W., and Baker, B. L.: The Local Action of Adrenocortical Steroids on Epidermis and Connective Tissue of the Skin, Endocrinology **47**:234, 1950.

24. Behrman, H. T., and Goodman, J. J.: Skin Complications of Cortisone and ACTH Therapy, J. A. M. A. **144**:218, 1950.

25. Sprague, R. G.: Cortisone and ACTH: A Review of Certain Physiologic Effects and Their Clinical Implications, Am. J. Med. **10**:567, 1951.

26. Ingle, D. J.; Sheppard, R.; Evans, J. S., and Kuizenga, M. H.: Comparison of Adrenal Steroid Diabetes and Pancreatic Diabetes in Rat, Endocrinology **37**:341, 1945.

27. Wolfson, W. Q.; Beierwaltes, W. H.; Robinson, W. D.; Duff, I. F.; Jones, J. R.; Knorpp, C. T., and Eya, M.: Corticogenic Hypothyroidism: Its Regular Occurrence and Clinical Significance During Prolonged Therapeutic Administration of ACTH or Cortisone, J. Lab. & Clin. Med. **36**:1005, 1950.

28. Hardy, J. D.; Riegel, C., and Erisman, E. P.: Experience with Protein Bound Iodine (PBI): The Effect of ACTH and Cortisone on Thyroid Function, Am. J. M. Sc. **220**:290, 1950.

29. Liebow, A. A.; Hales, M. R.; Bloomer, W. E.; Harrison, W., and Lindskog, G. E.: Studies on the Lung After Ligation of the Pulmonary Artery: Anatomical Changes, Am. J. Path. **26**:177, 1950.

30. Ragan, C.: Effect of ACTH and Cortisone on Connective Tissue, in Conference on Connective Tissue, Transactions of the First Conference, April 24-25, 1950, New York, N. Y., edited by C. Ragan, Josiah Macy Jr. Foundation, 1951, p. 137.

31. Molomut, N.; Spain, D. M., and Haber, A.: The Effect of Cortisone on the Spleen in Mice, Proc. Soc. Exper. Biol. & Med. **73**:416, 1950.

PRIMARY CARCINOMA OF THE LUNG IN THE MALE VETERAN

A Study Based on 3,946 Consecutive Necropsies at the Veterans Administration Center, Los Angeles, from 1948 through 1952

K. M. EARLE, M.D.
GALVESTON, TEXAS

CARCINOMA of the lung occurs with alarming frequency at the Veterans Administration Center, Los Angeles. This Center includes the General Medical and Surgical Hospital (Wadsworth), the Neuropsychiatric Hospital (Brentwood), and the Domiciliary Unit. In the past five years the operating bed capacity of these three units has varied as follows: General Medical and Surgical Hospital, 1,449 to 1,366 beds; Neuropsychiatric Hospital, 2,109 to 2,025 beds, and the Domiciliary Unit, 3,388 to 3, 074 beds.

From 1948 through 1952, carcinoma of the lung accounted for 21.9% of all malignant neoplasms which were found at necropsy in male veterans (or 8.3% of all necropsies). This paper is a report of the incidence of all malignant neoplasms which were found in a series of 3,946 consecutive necropsies during this five-year period. All malignant neoplasms are reported in order to show the frequency of carcinoma of the lung as related to other types (Chart). The proportion of malignant neoplasms may be slightly higher in this Veterans Administration Center than in some others, because many cases of carcinoma are referred to this center from other regional facilities.

Many recent institutional reports have shown that carcinoma of the lung now occurs more frequently than carcinoma of the stomach.* Steiner, Butt, and Edmondson, from the Los Angeles County Hospital,[12] showed that carcinoma of the lung ranked fourth among malignant neoplasms from 1918 to 1949, but in one recent four-year period (1943-1946) it was first. Their report showed that during 1943-1946 carcinomas of the large intestine were also more frequent than carcinoma of the stomach. The question of whether the increase in lung cancer is real and absolute or only apparent and relative is well covered by these authors and is the subject of considerable controversy. This discussion will not be repeated here, but it is the aim of this paper to report the high incidence of carcinoma of the lung in the male veteran in relation to that of all other types of malignant neoplasm in a recent five-year series of necropsies.

Since carcinoma of the lung occurs five or six times as frequently in men as in women [4] and since almost all of the cases in our series were adult men, this report will be confined to adult men. It is interesting, however, that of the 45 women in the series, 39 had a malignant neoplasm, and 3 of these had primary bronchogenic carcinoma.

Formerly Instructor in Pathology, University of California School of Medicine at Los Angeles, and Resident in Pathology, Veteran's Administration Center, Los Angeles; now Associate Professor of Pathology and Neuropathology, University of Texas School of Medicine.

* References 1, 3, 4, 6, 9, and 12.

Various histological classifications have been proposed for primary carcinoma of the lung. Most workers now believe that all carcinomas of the lung are derived from bronchial epithelium †; hence the commonly used term bronchogenic carcinoma. However, there is a small group of lung tumors having histological and clinical features suggesting an origin from alveolar or terminal bronchiolar cells.‡ These are referred to as alveolar cell tumor, alveolar cell carcinoma, pulmonary adenomatosis, and various other names. This type of lung cancer resembles a disease of sheep known as jaagsiekte, which is probably due to a virus.[5] However, since the etiology and pathogenesis of human lung cancer is uncertain, this category is not universally accepted.

In this institution, carcinomas of the lung have been classified into three major groups and a small fourth group as follows: (1) epidermoid if a squamous cell pattern predominates, (2) adenocarcinoma if a glandular pattern predominates, (3) undifferentiated if the cells are anaplastic and pleomorphic and lack a defi-

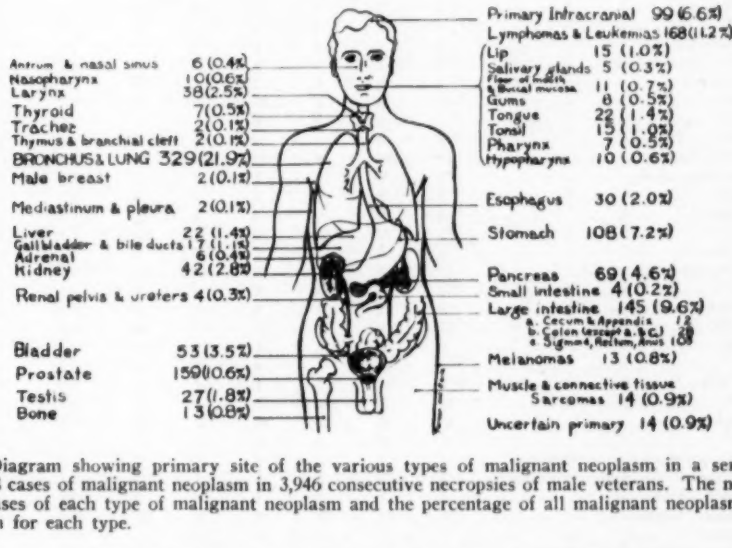

Primary Intracranial 99 (6.6%)
Lymphomas & Leukemias 168 (11.2%)
Lip 15 (1.0%)
Salivary glands 5 (0.3%)
Floor of mouth & Buccal mucosa 11 (0.7%)
Gums 8 (0.5%)
Tongue 22 (1.4%)
Tonsil 15 (1.0%)
Pharynx 7 (0.5%)
Hypopharynx 10 (0.6%)
Antrum & nasal sinus 6 (0.4%)
Nasopharynx 10 (0.6%)
Larynx 38 (2.5%)
Thyroid 7 (0.5%)
Trachea 2 (0.1%)
Thymus & branchial cleft 2 (0.1%)
BRONCHUS & LUNG 329 (21.9%)
Male breast 2 (0.1%)
Mediastinum & pleura 2 (0.1%)
Liver 22 (1.4%)
Gallbladder & bile ducts 17 (1.1%)
Adrenal 6 (0.4%)
Kidney 42 (2.8%)
Renal pelvis & ureters 4 (0.3%)
Esophagus 30 (2.0%)
Stomach 108 (7.2%)
Pancreas 69 (4.6%)
Small intestine 4 (0.2%)
Large intestine 145 (9.6%)
a. Cecum & Appendix 12
b. Colon (except a. & c.) 38
c. Sigmoid, Rectum, Anus 105
Bladder 53 (3.5%)
Prostate 159 (10.6%)
Testis 27 (1.8%)
Bone 13 (0.8%)
Melanomas 13 (0.8%)
Muscle & connective tissue Sarcomas 14 (0.9%)
Uncertain primary 14 (0.9%)

Diagram showing primary site of the various types of malignant neoplasm in a series of 1,498 cases of malignant neoplasm in 3,946 consecutive necropsies of male veterans. The number of cases of each type of malignant neoplasm and the percentage of all malignant neoplasms are given for each type.

nite pattern, (4) mixed types if areas resembling two of the above groups are found without definite predominance of either one. Reports from some institutions indicate that epidermoid carcinoma is the predominate type, whereas others find a predominance of the undifferentiated type.[2] All agree that the adenocarcinoma type is the least frequent. These differences may be due to slightly different criteria for classification.

Most authors agree that the right lung, which is larger, is the primary site of carcinoma more often than the left.[4] The upper lobes are said to be involved more frequently than the lower lobes,[11] a fact which makes bronchoscopic visualization difficult in over half the cases. This series confirms these two observations.

† References 2 and 4.
‡ References 7, 8, and 10.

In this necropsy series many of the cases had a lobectomy or pneumonectomy prior to death, and the exact site of the primary lesion was obtained from the surgical pathology report or from the history. The exact primary site was difficult to determine in many cases owing to the extensive spread of the tumor by the time of death. In some cases the tumor was so extensive that the exact lobe of origin or even the exact side was impossible to determine. These cases have been listed as uncertain in the Tables.

TABLE 1.—*Number of Each Type of Malignant Neoplasm in a Series of 1,498 Cases from a Series of 3,946 Consecutive Autopsies**

Primary Site of Malignant Neoplasm	No. of Cases						% of All Malignant Neoplasms	% of All Necropsies
	1948	1949	1950	1951	1952	Total		
Lip	4	5	0	4	2	15	1.0	0.38
Salivary glands	2	1	1	1	0	5	0.3	0.13
Mouth, gums	1	5	4	8	1	19	1.3	0.48
Tongue	2	5	7	0	8	22	1.5	0.55
Tonsil	4	3	3	2	3	15	1.0	0.38
Hypopharynx	1	2	0	4	3	10	0.7	0.25
Antrum, nasal sinuses	3	1	2	0	0	6	0.4	0.15
Nasopharynx	2	3	0	1	4	10	0.7	0.25
Larynx	1	10	9	13	5	38	2.5	0.96
Thyroid	1	2	0	3	1	7	0.5	0.18
Trachea	0	1	1	0	0	2	0.1	0.05
Bronchus, lung	49	59	69	83	69	329	21.9	8.33
Mediastinum, pleura	1	0	0	1	0	2	0.1	0.05
Pharynx	0	2	2	3	0	7	0.5	0.18
Esophagus	7	7	6	4	6	30	2.0	0.76
Stomach	15	20	23	26	24	108	7.2	2.73
Small intestine	2	0	0	0	2	4	0.3	0.10
Large intestine	17	30	24	28	46	145	9.7	3.67
Liver	5	1	75	3	6	22	1.5	0.55
Gallbladder, bile ducts	0	1	6	6	4	17	1.1	0.43
Pancreas	11	16	17	16	9	69	4.6	1.74
Adrenal	0	3	1	2	0	6	0.4	0.15
Kidney	8	11	6	4	13	42	2.8	1.06
Renal pelvis, ureters	0	0	0	3	1	4	0.3	0.10
Bladder	14	9	14	12	4	53	3.5	1.34
Prostate	13	35	36	40	35	159	10.6	4.02
Testis	9	6	2	6	4	27	1.8	0.68
Bone, synovial membrane (sarcomas)	5	2	2	0	4	13	0.9	0.32
Muscle, connective tissue (sarcomas)	2	2	2	4	4	14	0.9	0.35
Melanoma (malignant)	2	0	3	3	5	13	0.9	0.32
Lymphoma, leukemia	26	27	37	49	29	168	11.2	4.25
Male breast	0	1	1	0	0	2	0.1	0.05
Thymus, branchial cleft carcinoma	0	0	1	0	1	2	0.1	0.05
Primary intracranial malignant tumors	16	22	25	17	19	99	6.6	2.50
Uncertain primary	3	5	0	2	4	14	0.9	0.35
Totals	226	297	311	348	316	1,498		

* All were male veterans. The percentage of all malignant neoplasms and percentage of all autopsies is given for each group.

THE NECROPSY SERIES

From 1948 through 1952 there were 3,946 consecutive necropsies at the Veterans Administration Center, Los Angeles. During this period the autopsy percentage varied from 70 to 84% for an average of 78.8%. Of these necropsies, 3,901 were men, and only 45 were women. One or more malignant neoplasms (37.9%) were found in 1,498 men. Forty-four cases had more than one primary malignant neoplasm. These are listed only as the type which is most malignant from the clinical and pathological standpoint.

The figures in Tables 1 and 2 are based on findings in 1,498 men who had malignant neoplasms. The small number of women are not included. The incidence

TABLE 2.—*Number of Cases in Each Decade of Life for Each Type of Malignant Neoplasm in a Series of 1,498 Cases*

Primary Site of Malignant Neoplasm	No. of Cases in Each Decade of Life									Av. Age, Yr.
	10 to 20	20 to 30	30 to 40	40 to 50	50 to 60	60 to 70	70 to 80	80 to 90	90 to 100	
Lip	..	..	1	1	5	5	3	..	..	60.33
Salivary gland	..	..	..	..	2	1	1	1	..	69.40
Mouth, gums										
Floor of mouth, buccal mucosa	..	..	1	1	5	1	1	2	..	59.00
Gums	..	..	..	..	7	..	..	1	..	59.25
Tongue	..	..	..	2	8	8	3	1	..	61.13
Hypopharynx	..	..	..	1	5	3	1	..	..	59.25
Tonsil	..	..	..	2	9	1	3	..	..	57.66
Antrum, nasal sinus	..	..	..	1	..	2	3	..	..	66.50
Nasopharynx	..	..	..	3	4	2	1	..	..	56.00
Larynx	..	..	1	1	17	10	7	2	..	61.15
Thyroid	..	..	..	1	2	2	1	1	..	60.85
Trachea	..	..	..	1	1	..	..	..	..	52.50
Bronchus, lung	..	3	11	35	152	93	23	2	..	57.63
Mediastinum, pleura	..	1	..	..	1	..	..	..	..	41.50
Pharynx	..	..	..	..	3	4	..	..	..	60.57
Esophagus	..	..	..	1	12	6	10	1	..	63.46
Stomach	..	2	2	8	29	38	22	6	1	61.18
Small intestine	..	..	..	..	1	3	..	..	..	61.75
Large intestine										
Cecum, appendix	..	..	..	..	3	5	2	2	..	66.75
Colon (except cecum, sigmoid)	..	..	2	..	12	4	7	2	1	63.17
Sigmoid, rectum, anus	..	3	5	6	37	28	23	3	..	59.02
Liver	..	..	..	5	11	4	1	1	..	56.40
Gallbladder, bile ducts	..	..	1	..	5	5	6	..	..	62.52
Pancreas	..	1	2	6	32	10	13	5	..	60.31
Adrenal	..	..	1	5	..	..	..	..	..	49.33
Kidney	..	..	1	4	17	12	6	2	..	60.07
Renal pelvis, ureter	..	..	..	..	2	2	..	..	..	66.25
Bladder	..	..	..	2	22	14	12	3	..	62.32
Prostate	..	..	..	..	30	41	55	30	3	69.78
Testis	..	13	9	4	..	1	..	..	..	32.11
Bone, synovial membrane (sarcomas)	1	5	2	2	2	1	..	..	..	37.00
Muscle, connective tissue (sarcomas)	..	3	1	..	7	1	2	..	..	50.50
Melanoma (malignant)	..	2	2	1	3	3	1	1	..	52.00
Lymphoma, leukemia										
Myelogenous leukemia	1	8	6	3	4	4	..	..	..	38.73
Lymphatic leukemia	..	4	2	3	5	4	2	1	..	49.80
Monocytic, stem-cell leukemia	..	1	2	..	2	2	1	..	..	49.25
Lymphosarcoma	..	1	4	1	9	4	1	..	..	51.90
Reticulum cell sarcoma	..	1	6	4	5	1	2	1	..	49.50
Plasma cell myeloma	..	..	2	4	7	7	5	2	..	59.96
Hodgkin's disease	..	15	12	4	7	3	2	3	..	42.28
Male breast	..	..	..	1	..	1	..	..	..	56.32
Thymus, branchial cleft carcinoma	..	..	..	..	..	1	1	..	..	67.00
Primary intracranial malignant tumors	..	..	9	23	11	33	15	8	..	49.41
Uncertain primary	..	..	3	1	4	5	..	1	..	52.92
Totals	2	75	100	124	522	357	239	74	5	57.83

of each type of malignant neoplasm is reported on a yearly basis, as well as the five-year total, in order to see if carcinoma of the lung showed an actual or only a relative increase during this period.

Lymphomas and leukemias are included as malignant neoplasms, but squamous and basal cell carcinomas of the skin have been eliminated, because most of these

were localized lesions, and not every suspicious skin lesion was studied at autopsy. Malignant melanomas are included, if they showed metastasis. Carcinomas of the lip are included.

TYPES OF MALIGNANT NEOPLASMS

All of the general categories will be discussed in their various histological types and subdivisions.

The number of cases of each type of malignant neoplasm is shown in Table 1 on a yearly and a five-year basis, with the percentage of all malignant neoplasms and percentage of all necropsies which each group comprised.

The number of each type of neoplasm which occurred in each decade of life is shown in Table 2, with the average age for each group, total cases of all types in each decade, and the total average age for the series.

In the following discussion, the term epidermoid carcinoma is used instead of squamous cell carcinoma in accordance with International Standard Nomenclature.

Bronchus and Lung.—The following histological types were found in 329 cases:

Epidermoid carcinoma	135
Undifferentiated carcinoma	127
Adenocarcinoma	56
Mixed types	10
Fibrosarcoma	1

The following anatomical locations were found as primary sites:

Right lung	162
Left lung	144
Carina	4
Uncertain side	19

The following lobes were considered to be the primary sites:

Right upper lobe	67
Left upper lobe	70
Right middle lobe	14
Left lower lobe	34
Right lower lobe	31
Left main bronchus	32
Right main bronchus	39
Carina	4
Uncertain lobe (side was uncertain in these)	38

Lip.—All 15 of the cases were epidermoid (squamous cell) carcinomas.

Salivary Glands.—In the five cases, there were three tumors of the submaxillary gland (one adenocarcinoma, one epidermoid carcinoma, and one mixed tumor with extension), and two tumors of the parotid gland (one adenocarcinoma and one mixed tumor with recurrence after operation and metastasis to lung).

Mouth and Gums.—Of 19 cases, 11 were tumors of floor of mouth and buccal mucosa (all epidermoid carcinomas), and 8 were tumors of the alveolar ridge (all epidermoid carcinomas).

Tongue.—Of 22 cases, all were epidermoid carcinomas.

Tonsils.—Of 15 cases, all were epidermoid carcinomas (right tonsil 6, left tonsil 9).

Hypopharynx.—Of 10 cases, all were epidermoid carcinomas.

Antrum and Nasal Sinuses.—Of six cases, there were three epidermoid carcinomas of the antrum, two epidermoid carcinomas of the maxillary sinus, and one transitional cell carcinoma of the ethmoid sinus.

Nasopharynx.—Of 10 cases, there were 5 epidermoid carcinomas, 3 transitional cell carcinomas, and 2 adenocarcinomas.

Larynx.—Of 38 cases, 37 were epidermoid carcinomas, and 1 was a transitional cell carcinoma. The exact point of origin was given in some cases as follows: Epiglottis (3), vocal cord (1), piriform sinus (2), artenoid cartilage region (1), unspecified (31).

Thyroid.—Of seven cases, there were five adenocarcinomas, one mixed adenocarcinoma with squamous cell features, one undifferentiated type.

Trachea.—Epidermoid carcinoma was found in two cases.

Mediastinum and Pleura.—Of two cases, one was a mesothelial cell sarcoma, and one was a teratoma.

Pharynx.—Epidermoid carcinomas were found in seven cases.

Esophagus.—Of 30 cases, all were epidermoid carcinomas. The anatomical locations were given in some cases as follows: upper third (2), middle third (9), lower third (3), multicentric (1), not stated or too extensive to determine (15).

Stomach.—Of 108 cases, 104 were diagnosed as adenocarcinoma, undifferentiated carcinoma, carcinoma simplex, or scirrhous carcinoma. The remainder consisted of two lymphosarcomas, one plasmacytoma, and one leiomyosarcoma.

Small Intestine.—Of four cases, two were adenocarcinomas of the duodenum; one was adenocarcinoma of the jejunum, and one was leiomyosarcoma of the jejunum.

Large Intestine.—One hundred forty-five cases were subdivided as follows:

Cecum and Appendix: Of 12 cases, there were 11 adenocarcinomas of cecum and 1 adenocarcinoma of appendix.

Colon (Except Cecum and Sigmoid): Of 28 cases, all were adenocarcinomas. The anatomical locations were ascending colon (six), hepatic flexure (four), transverse colon (seven), splenic flexure (four) descending colon (four), uncertain site (three [resected elsewhere]).

Sigmoid, Rectum, and Anus: Of 105 cases, all were adenocarcinomas, except those of the anus (2 epidermoid carcinomas). The anatomical locations were given as follows: sigmoid colon (20), rectosigmoid (16), rectum (67), anus (2).

Liver.—Of 22 cases, there were 21 hepatocellular carcinomas and 1 angiosarcoma.

Gallbladder and Bile Ducts.—Of 17 cases, the gallbladder was involved in 7 cases ‡ (5 adenocarcinomas and 2 undifferentiated carcinomas), and the bile ducts were involved in 10 cases (all adenocarcinomas). Of the bile duct carcinomas, the locations were ampulla (three), common duct (two), cystic duct (one), intrahepatic ducts (two), exact site undetermined (nine).

Pancreas.— Of 69 cases, all were carcinomas. Anatomical locations were head of pancreas (21), body or tail (27), uncertain exact origin (21).

Adrenal.—Of six cases, one pheochromocytoma, four carcinomas, and one neuroblastoma were found.

Kidney.—Of 42 cases, there were 41 carcinomas and 1 fibrosarcoma. Anatomical locations were right kidney (23) and left kidney (19).

Renal Pelvis and Ureters.—All of the four cases were transitional cell carcinomas. Two were in the renal pelvis, and two were in the ureter.

Bladder.—Of 53 cases, 45 were transitional cell carcinomas, 7 were epidermoid carcinomas, and 1 was a leiomyofibrosarcoma.

Prostate.—Of 159 cases, all were carcinomas. Sixty-five of these cases showed definite extension or metastasis, and 94 cases showed only focal adenocarcinoma (in situ, occult).

Testis.—Of 27 cases, there were 6 seminomas, 10 embryonal carcinomas, 5 teratocarcinomas, 2 choriocarcinomas, 3 teratomas with malignant features, and 1 lymphosarcoma. Anatomical locations were right testicle (10), left testicle (12), bilateral (1) and, uncertain or not stated (4).

Bone and Synovial Membrane.—Of 13 cases, 6 were osteogenic sarcoma, 4 were fibrosarcoma, 2 were Ewing's sarcoma, and 1 was synovial sarcoma.

Muscle and Connective Tissue.—Of 14 cases, there were 4 fibrosarcomas, 3 liposarcomas, 1 leiomyosarcoma, 1 myxosarcoma, 2 rhabdomyosarcomas, and 3 undifferentiated sarcomas.

Melanomas, (Malignant).—The antomical sites of origin in 13 cases were eye (1), foot (3), axilla (2), scalp (1), inguinal region (1), preauricular region (1), postauricular region (1), nose (1), and uncertain (2).

Lymphomas and Leukemias.—One hundred sixty-eight cases were subdivided as follows: myelogenous leukemia (26), lymphatic leukemia (21), monocytic and stem cell leukemias (8), lymphosarcoma (20), reticulum cell sarcoma (20), plasma cell myeloma (27), and Hodgkin's disease (46).

Male Breast.—The two cases were adenocarcinomas.

Thymus and Branchial Cleft.—One epidermoid carcinoma of branchial cleft origin and one carcinoma of thymus comprised the two cases.

Primary Intracranial Tumors.—Of 99 cases, there were 48 cases of glioblastoma multiforme (astrocytoma, grade 3 or 4), 12 astrocytomas (grade 1 or 2), 4 oligodendrogliomas, 1 spongioblastoma polare, 1 subependymal glioma, 1 neuroepithelioma, 5 gliomas unclassified, 1 pinealoma, 11 meningiomas, 1 meningeal sarcoma, 2 ependymomas, 1 carcinoma of the choroid plexus, 1 craniopharyngioma, 1 cholesteatoma, 1 paraphyseal cyst, 3 hemangiomas, 3 hemangioblastomas, 2 chordomas.

Uncertain Primary.—Five of 14 cases were epidermoid carcinomas, and 9 were undifferentiated and anaplastic malignant neoplasms.

COMMENT

If carcinomas of the skin are excluded, carcinoma of the lung is the most frequent malignant neoplasm which is encountered in autopsies of male veterans at the Veterans Administration Center, Los Angeles (Chart). From 1948 through 1952, approximately 1 out of every 12 autopsies was a case of primary carcinoma of the lung (8.33% of all autopsies). Approximately one out of every five malignant neoplasms of the male veteran was a case of primary carcinoma of the lung (21.9% of all malignant neoplasms).

Histologically, epidermoid carcinoma was the most frequent type; undifferentiated carcinoma was second, and adenocarcinoma was third in frequency. The right lung was the primary site slightly more frequently than the left lung. The primary site was in the upper lobes in 41% of the cases.

The high incidence of carcinoma of the lung in this series was apparent in each of the five years studied (Table 1). This type was the commonest malignant neoplasm in each of the five years, carcinoma of the skin excluded. The other types of tumors maintained approximately the same relative frequency in each of the five years. Carcinoma of the large intestine was more frequent than carcinoma of the stomach in each of the five years.

Lymphomas and leukemias, if taken as a group, composed the second most frequent type of malignant neoplasm (4.2% of all autopsies or 11.2% of all malignant neoplasms). Carcinoma of the prostate is third in frequency (4.02% of all autopsies or 10.6% of all malignant neoplasms).

Carcinoma of the large intestine ranks fourth in frequency (3.6% of all autopsies or 9.7% of all malignant neoplasms). Carcinomas of the sigmoid, rectum, and anus accounted for slightly over two-thirds of this group.

Carcinoma of the stomach ranks only fifth in frequency in this series (2.73% of all autopsies or 7.2% of all malignant neoplasms). Therefore, carcinoma of the stomach is far less frequent here than would have been predicted from statistics elsewhere, but carcinoma of the lung is now more frequent than carcinoma of the stomach in several recent reports, as pointed out in the introduction.

Primary intracranial tumors rank sixth in frequency (2.50% of all autopsies or 6.6% of all malignant neoplasms). Glioblastoma multiforme was the most frequent tumor in this group.

Carcinoma of the pancreas ranks seventh (1.74% of all autopsies or 4.6% of all malignant neoplasms). Carcinoma of the bladder ranks eighth (1.34% of all autopsies or 3.5% of all malignant neoplasms). Carcinoma of the kidney ranks ninth (1.06% of all autopsies or 2.8% of all malignant neoplasms). Carcinoma of the larynx ranks 10th (0.96% of all autopsies or 2.5% of all malignant neoplasms).

None of the remaining tumor groups accounted for more than 2% of all malignant neoplasms.

The average age for patients with carcinoma of the lung was 56.63 years (Table 2). The youngest was 25, and the oldest was 83 years of age. The average age for all cases of malignant tumor in the series was 57.83 years. The youngest case in the series was 18, and the oldest case was 99 years of age.

SUMMARY

In a series of 3,946 consecutive necropsies at the Veterans Administration Center, Los Angeles, from 1948 through 1952, there were 1,498 cases with one or more malignant neoplasms. Carcinomas of the skin (except malignant melanoma and carcinoma of the lip) are not included in this figure.

Primary carcinoma of the lung was found in 329 of these cases (21.9% of all malignant neoplasms and 8.33% of all autopsies). This was the commonest type of malignant neoplasm. Epidermoid carcinoma was the most frequent type; undif-

ferentiated carcinoma was second, and adenocarcinoma was third in frequency The right lung was the primary site oftener than the left. The upper lobes were oftener the primary site than the lower lobes.

The high incidence of carcinoma of the lung maintained its high frequency, relative to the other types, in each of the five years studied. The average age for patients with carcinoma of the lung was 57.63 years. The youngest case was 25, and the oldest was 83 years of age. The average age for all patients with malignant tumors was 57.83 years for this series.

The following groups of malignant neoplasms ranked 2nd and 10th in order of frequency: lymphomas and leukemias, carcinoma of the prostate, carcinoma of the large intestine (mostly sigmoid and rectum), carcinoma of the stomach, primary intracranial tumors, carcinoma of the pancreas, carcinoma of the bladder, carcinoma of the kidney, carcinoma of the larynx.

Encouragement and assistance in the preparation of this paper was given by Dr. S. C. Madden, Professor of Pathology, University of California School of Medicine at Los Angeles; Dr. Leo Kaplan, Chief of Laboratory Service, Veteran's Administration Center, Los Angeles; Miss Martha S. Wolking, secretary to Dr. Kaplan; Mrs. Cleo B. Nelson, R.R.L., Chief Medical Record Librarian of the Veteran's Administration Center; Mrs. Louise V. McKelvey, Department of Pathology, University of California at Los Angeles, who typed the manuscript, and Mr. Lloyd Turner, who assisted with the Chart.

REFERENCES

1. Abrams, H. L.; Spiro, R., and Goldstein, N.: Metastases in Carcinoma: Analysis of 1,000 Autopsied Cases, Cancer **3**:74-85, 1950.

2. Anderson, W. A. D., Editor, Pathology, St. Louis, C. V. Mosby Company, 1948.

3. Ariel, I. M.; Avery, E. E.; Kanter, L.; Head, J. R., and Langston, H. T.: Primary Carcinoma of the Lung: A Clinical Study of 1,205 Cases, Cancer **3**:229-239, 1950.

4. DeBakey, M. E.; Ochsner, A., and DeCamp, P. T.: Primary Carcinoma of the Lung, Surgery **32**:877-891, 1952.

5. Dungal, N.: Experiments with Jaagsiekte, Am. J. Path. **32**:737-759, 1946.

6. Graham, E. A.: Primary Cancer of the Lung with Special Consideration of Its Etiology (Fifth James Ewing Memorial Lecture), Bull. New York Acad. Med. **27**:261-274, 1951.

7. Herbut, P. A.: Bronchiolar Origin of "Alveolar Cell Tumor" of the Lung, Am. J. Path. **20**:911-929, 1944.

8. Ikeda, K.: Alveolar Cell Carcinoma of the Lung, Am. J. Clin. Path. **15**:50-63, 1945.

9. McSwain, B., and Williams, R. B., Jr.: The Relative Frequency of Cancer, Cancer **4**:416-422, 1951.

10. Neubuerger, K. T., and Geever, E. F.: Alveolar Cell Tumor of the Human Lung, Arch. Path. **33**:551-569, 1942.

11. Ochsner, A.; DeCamp, P.; DeBakey, M. E., and Ray, C. J.: Bronchogenic Carcinoma: Its Frequency, Diagnosis, and Early Treatment, J. A. M. A. **148**:691-697, 1952.

12. Steiner, P. E.; Butt, E. M., and Edmondson, H. A.: Pulmonary Carcinoma Revealed at Necropsy, with Reference to Increasing Incidence in Los Angeles County Hospital, J. Nat. Cancer Inst. **11**:497-510, 1950.

AN ACID-FAST LIPID FROM SPERMATOZOA

J. W. BERG, M.D.
NEW YORK

IN DESCRIBING the tissue response to extravasated spermatozoa, Friedman and Garske [1] emphasized the resemblance between the resulting granulomas and tubercles. They described not only foci of epithelioid and giant cells but also regions of caseation. No reason was advanced for the stimulation of this particular type of tissue reaction by sperm, and it was concluded that "the factors leading to the development of spermatic granulomas . . . are not clearly understood."

The same can no longer be said for the tissue reactions to tubercle bacilli themselves. Sabin and her colleagues [2] have succeeded in assigning to various chemical fractions of the bacilli the responsibilities for the various tissue responses which together make up the tubercle. By analogy it might be considered possible that similar compounds (especially lipids) might be responsible for producing the same tissue responses in spermatic granulomas.

Recently, sperm and mycobacteria have been shown to share another property, that of acid-fastness.* Examination of this staining reaction showed that in sperm, as in lepra bacilli, acid-fastness was not related to cell structure. Rather, it was altered only by treatment which also altered the chemical composition of the cells. This type of acid-fastness was the same qualitatively and quantitatively as that shown by mycolic and leprosinic acids, two long-chain hydroxy fatty acids isolated from tubercle and lepra bacilli, respectively. It was therefore suggested that the staining reaction in these bacilli might be attributed to the presence of these acids. Then, again by analogy, spermatozoa, staining in the same manner as mycobacteria, might also possess lipids of the mycolic acid type.

Reports in the literature of chemical analyses of sperm † describe no compound to which acid-fastness could be ascribed in the light of present knowledge. On the other hand, it appeared quite probable that if, for instance, mycolic acid itself had been present in sperm, it would not have been found by the methods of analysis described. Although in its pure state mycolic acid is, like other lipids, soluble in chloroform, it cannot be removed from the bacterial cells by chloroform, acetone, or alcohol-ether extraction.[10] Rather, preliminary acid hydrolysis is necessary before the lipid character of the acid becomes manifest. The lipids studied in

Postdoctorate Fellow, National Cancer Institute.
Present address: Department of Pathology, Memorial Center.
Aided by a grant from the National Cancer Institute of the United States Public Health Service.
Formerly from the Department of Pathology, Yale University School of Medicine, New Haven, Conn.

* References 3 and 4.

† References 5 to 9.

spermatozoa were only those removed by acetone and chloroform. Acid hydrolyses were done, but the extracts were analyzed only for nonlipid compounds. Hence it appeared quite logical to study the chemistry of sperm once again, this time looking specifically for lipid of the mycolic acid type. This paper reports the results of such a search, the finding of a previously undescribed lipid fraction which is acid-fast and capable of inducing a granulomatous tissue response.

MATERIALS AND METHODS

Most of the preliminary extraction studies were done on human sperm washed from one to three pairs of epididymides obtained as surgical or autopsy specimens. Repetitive and final studies were then made on single semen specimens from healthy adults, where the centrifuged volume of washed sperm was about 0.5 cc. The epididymal washings contained a few immature germ cells on microscopic examination, the semen specimens none. For the latter definitive procedure, the semen specimen after liquefication was diluted to a volume of 10 cc. with 0.85% saline. This cell suspension was centrifuged, and the supernatant fluid was discarded. The sperm was then washed in two 8-cc. amounts of 0.85% saline and two similar volumes of distilled water. Each time the cells were well mixed with the solution and then separated by centrifugation. After the washing with water the cells were still strongly acid-fast. (Acid-fastness was defined [11] as resistance to five-minute differentiation in 5% acetic acid in alcohol after staining for 15 minutes in saturated crystal violet buffered to pH 7.8.)

After washing, soluble lipids were removed by three extractions with 8 cc. of acetone (4, 4, and 16 hours of frequent agitation) and a similar course of treatment with equal parts of ether and absolute alcohol. The cells were still acid-fast after this treatment, and evaporation of the extracting fluids left no acid-fast material. The cells were rehydrated through absolute, 95%, and 75% alcohol. The bulk of nucleoprotein was then removed by incubation of the sperm for 24 hours in 10 cc. of 1 N NaCl at 60 C. At the end of this time, after washing with water as before, the cells were Feulgen-negative but still acid-fast. Finally the acid-fast material was extracted (together with many other substances) by suspension for two hours in 8 cc. of 1 N HCl at 60 C. Paper chromatography of the filtrate of this extraction showed a strong acid-fast band. Actual purification, however, was done in three steps. First, protein was precipitated from the filtered HCl solution by dropwise addition of 5% phosphotungstic acid. The precipitate, when thoroughly washed with 1 N HCl, showed no residual acid-fastness.

Once the precipitated protein had been removed by filtration, the HCl solution was layered over an equal volume of chloroform in a separatory funnel. The solutions were mixed well by frequent shakings. After 24 hours, paper chromatography showed all the acid-fast material in the chloroform layer, none in the acid layer. The two layers were separated and the acid-fast material was then precipitated from the chloroform by addition of 2 volumes of absolute alcohol, as had been described for the purification of mycolic acid.[10] After precipitation, the solution was filtered, and the filtrate was discarded. The precipitate was washed in absolute alcohol and then redissolved in 5 cc. of chloroform. It was reprecipitated by alcohol, as before.

Control extractions of the same type were done on minced liver, spleen, and epididymides containing no sperm. In no case was alcohol-precipitable lipid found.

PROPERTIES OF THE EXTRACTED MATERIAL ‡

After five reprecipitations the dried material was white, noncrystalline, and waxy in appearance. It melted at 123 to 127 C., and this was not changed by further precipitations. It was readily soluble in chloroform and acetone. It was slightly soluble in 5% NaOH. It appeared to be insoluble in water, acids, $NaHCO_3$, alcohol, ether, benzene, and carbon tetrachloride.

‡ The mycolic and leprosinic acids used in the following procedures for comparison with the unknown lipid were made available by Dr. E. Mylon, of the Department of Pathology, Yale University School of Medicine.

Because the amount available for chemical tests was only about 10 mg., most of the tests listed below were carried out as spot tests on portions of lipid adsorbed on filter paper.§ In addition to being acid-fast, the material was sudanophilic.‖ It did not reduce potassium permanganate. The biuret, Millon, xanthoproteic, and Hopkins-Cole tests for protein and amino acids were negative, as were tests for glutathione, cystine, and purines and pyrimidines. Tests for iron, calcium, phosphate, and sulfur were also negative. No cholesterol was found.

Except that this compound had a higher melting point, the results of these tests were the results given also by mycolic and leprosinic acids. Because most of these

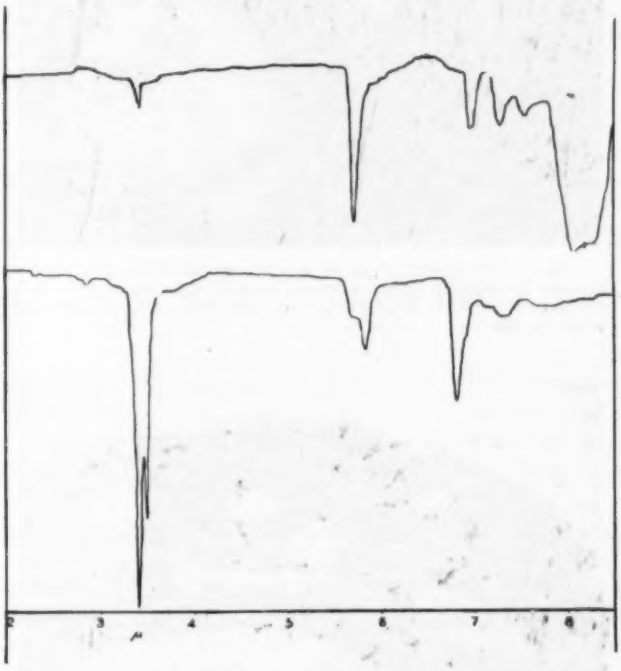


Fig. 1.—Infrared absorption spectrum of the acid-fast lipid fraction from spermatozoa (upper curve) compared with that of mycolic acid (lower curve). Substances in chloroform solution.

tests were negative, more positive evidence of a relationship was sought by a comparison of the molecular (infrared) spectra of the acid-fast compound from sperm and of mycolic acid.

Figure 1 shows the similarity between the spectra of mycolic acid and of the unknown lipid. Both substances show definite absorption bands at about 3.4, 5.8, 6.9, and 7.3μ. The main difference between the two spectra is that the unknown

§ The materials were studied in chloroform solution.

‖ The actual determinations were carried out for the author by J. F. Riley, of the Department of Chemistry, Yale University.

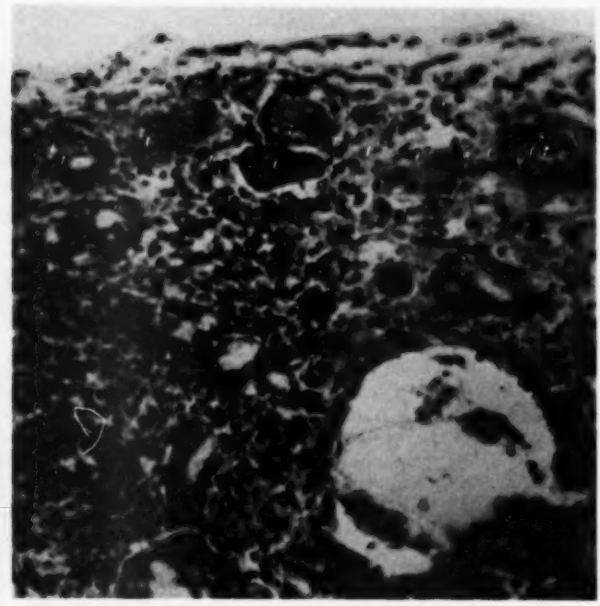


Fig. 2.—Granuloma produced in the subcutaneous tissues of a hamster by injection of the acid-fast material from sperm nine days previously. Note the large number of giant cells. Hematoxylin and eosin; × 300.

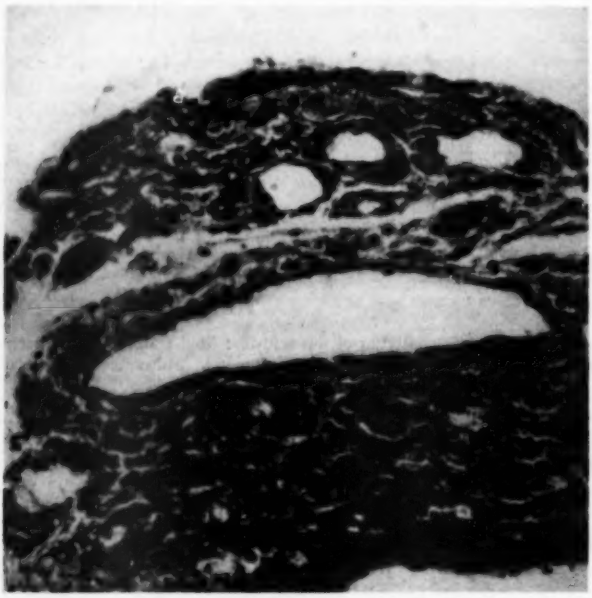


Fig. 3.—Granuloma produced in the same animal by injection of mycolic acid nine days previously. Again there are numerous giant cells, and the two reactions are almost identical in appearance. Hematoxylin and eosin; × 300.

lipid shows, in addition, a broad and strong region of absorption from 8.1 to 8.35μ. The location of the absorption bands of mycolic acid correspond to what is known of the structure of this compound, and it is possible (see Comment) to apply the same criteria to the spectrum of the unknown lipid.

Finally, the reaction of tissues to the unknown lipid was tested. Dry lipid was taken up in a trocar and injected into the subcutaneous tissue of a hamster. Mycolic acid was introduced in the same manner on the opposite side of the animal. The hamster was killed after nine days. Figures 2 and 3 show the similarity of the histological appearance of the two lesions. In both polymorphonuclear leucocytes, lymphocytes, and histiocytes were present in large numbers. Less numerous but perhaps more specific were the plasma cells, epithelioid cells, and giant cells. There was no necrosis. Foam cells and eosinophiles were not seen. When the ages of the lesions were taken into account, the picture was quite similar to that described for the spermatic granulomas.[1]

COMMENT

These results then appear to confirm the predictions about the chemical composition of sperm derived from their staining and the type of inflammatory response engendered by their presence. Both reactions, not typical of most cells, could be duplicated by using lipid fractions from the sperm just as they could by similar fractions from tubercle bacilli.

The term "lipid fraction" has been used advisedly. Sabin and Joyner [12] felt that polysaccharide impurities accompanying the mycolic acid fractions which they tested were responsible for the massive neutrophile response which they encountered. A similar response was encountered here and therefore may be explained in the same way. Even without such evidence of impurity, however, the simplicity (crudity?) of the methods utilized here was such as to remove little, if any, of the various substances which had been found with mycolic acid under similar circumstances.[10]

Similar assumptions of impurity serve to explain the differences between the infrared spectra of the unknown lipid and the model mycolic acid. When the spectrum of mycolic acid is compared with that of other compounds,[13] it appears that the absorptions at 3.4, 6.8, and 7.3μ may be ascribed to the presence of the aliphatic chain with its C-C and C-H bonds. The absorption at 5.8μ corresponds to the presence of the carboxyl group of the molecule. All these bands are present in the spectrum of the spermatic lipid, and by analogy it may also be said to be aliphatic and to possess a carboxyl group. However, the broad peak above 8μ for the unknown compound corresponds to absorption seen in many cases of esterification, and the slight shift in the location of the main carboxyl peak also corresponds to the presence of an ester, rather than the free radical. The curves here appear inadequate to give information about the presence or absence of a hydroxyl radical. Such a group is postulated for mycolic acid. If present, it should absorb in the region between 2 to 3μ. There is only a slight irregularity in this region, however, for mycolic acid. Since the solution of the unknown was even more dilute, the absence of specific hydroxyl absorption gives no positive information. Thus all we can deduce about the structure of the unknown lipid is that it possesses an infrared spectrum compatible with the ester of an aliphatic acid similar mycolic acid.

SUMMARY AND CONCLUSIONS

After previously described lipids had been removed from spermatozoa by chloroform extraction, a second lipid fraction was released from these cells by acid hydrolysis. This second fraction carried the acid-fastness previously noted in intact sperm. In tissues it produced reactions similar to spermatic granulomas. It resembled mycolic acid in its molecular (infrared) absorption spectrum and in the tissue reaction which it produced.

Advice and help in the preparation of this paper was given by Dr. H. Bunting, Department of Pathology, in whose laboratory this work was done, and by Dr. C. A. Hargreaves, Department of Chemistry.

REFERENCES

1. Friedman, N. B., and Garske, G. L.: Inflammatory Reactions Involving Sperm and the Seminiferous Tubules: Extravasation, Spermatic Granulomas, and Granulomatous Orchitis, J. Urol. **62**:363-374, 1949.

2. Sabin, F. R.: Cellular Reactions to Fractions from Tubercle Bacilli, Am. Rev. Tuberc. **44**:415-423, 1941.

3. Berg, J. W.: Differential Staining of Spermatozoa in Sections of Testis, Am. J. Clin. Path. **23**:513-515, 1953.

4. Berg, J. W.: The Dual Nature of Acid-Fastness, Yale J. Biol. & Med., to be published.

5. Zittle, C. A., and O'Dell, R. A.: Chemical Studies of Bull Spermatozoa: Lipid, Sulfur, Cystine, Nitrogen, Phosphorus, and Nucleic Acid Contents of Whole Spermatozoa and Parts Obtained by Physical Means, J. Biol. Chem. **140**:899-907, 1941.

6. Porter, J. C.; Shankman, S., and Melampy, R. M.: Chemical Composition of Bovine Spermatozoa, Proc. Soc. Exper. Biol. & Med. **77**:53-56, 1951.

7. Slovtzov, B.: Sur la composition biochemique de liquide spermatique, Compt. rend. Soc. biol. **79**:208, 1916.

8. Wagner-Jauregg, T.: Über das Vorkommen des Heptakosans im menschlichen Sperm, Ztschr. physiol. Chem. **269**:56-58, 1941.

9. Schmidt-Nielsen, S., and Sundsvold, O. C.: Über den Fett- und Cholesteringehalt der Fischmilch, Kong. norske vidensk. selsk. forh. **16**:59-60, 1943.

10. Stodola, F. H.; Lesuk, A., and Anderson, R. J.: The Chemistry of the Lipids of Tubercle Bacilli: The Isolation and Properties of Mycolic Acid, J. Biol. Chem. **126**:505-513, 1938.

11. Berg, J. W.: Acid-Fastness as a Histochemical Reaction, J. Histochem. & Cytochem. **1**:436-441, 1953.

12. Sabin, F. R., and Joyner, A. L.: Cellular Reactions to Defatted Tubercle Bacilli and Their Products, J. Exper. Med. **68**:853-868, 1938.

13. Barnes, R. B.; Gore, R. C.; Liddel, U., and Williams, V. Z.: Infrared Spectroscopy: Industrial Applications and Bibliography, New York, Reinhold Publishing Corporation, 1944.

CARCINOMA OF THE TESTES METASTATIC FROM CARCINOMA OF THE PROSTATE

SAUL KAY, M.D.
G. R. HENNIGAR, M.D.
AND
J. W. HOOPER Jr., M.D.
RICHMOND, VA.

TO THE best of our knowledge, only four cases of metastatic carcinoma of the testis following primary carcinoma of the prostate have been reported in the literature.* Undoubtedly this is a rare event and is not mentioned in Willis' [5] revised edition of "The Spread of Tumours in the Human Body." The infrequent observation of prostatic metastases to the testis may, in part, be explained by the discarding of the testicular tissue removed in the operating room as a therapeutic measure for prostatic carcinoma. Such had been the practice for a long time at this institution, and probably in many other hospitals testicular tissue may have been discarded without submitting it for pathological examination.

Willis' [5] revised edition of "The Spread of Tumours in the Human Body." The retrograde extension of embolism in spermatic veins, retrograde lymphatic extension, and arterial embolism. Theoretically a tumor in the genitourinary tract might also descend by way of the ejaculatory duct to reach the testis. It is the purpose of this paper to present three cases of testicular carcinoma following carcinoma of the prostate and to propose one theory of pathogenesis as exemplified by one of the cases.

REPORT OF CASES

CASE 1.—S. W., a 68-year-old Negro man, was admitted to the St. Philip Hospital on May 12, 1952, complaining of pains in the lower lumbar region and legs. He was well until 1947, when he developed marked urinary frequency. His trouble was related to the prostate, for which he was admitted on six occasions at another hospital, from Sept. 15, 1947, to Nov. 22, 1949. During these admissions transurethral resections were done, and a diagnosis of carcinoma of the prostate was rendered. Physical examination revealed tenderness over the lower lumbar vertebrae. The breasts were hypertrophied, and the liver was palpable 2 fingerbreadths beneath the costal margin. On rectal examination the prostate was enlarged three times its natural size and very hard. The remainder of the examination was noncontributory. Laboratory examination showed an elevated alkaline and acid phosphatase and positive serology for syphilis. Roentgenogram showed moth-eaten changes in the 12th thoracic and the first lumbar vertebra and in both iliac bones, which were compatible with metastatic carcinoma from a primary prostatic focus. The patient was treated with diethylstilbestrol (Stilbestrol) and was given x-ray therapy to the spine. A bilateral subcapsular orchidectomy was done (see pathological report below). He was discharged June 5, 1952, to be followed in the Genitourinary Clinic

From the Laboratory of Surgical Pathology, Department of Pathology, Department of Urology, Medical College of Virginia.

*References 1 to 4.

under diethylstilbestrol therapy. The last note in the Genitourinary Clinic (Feb. 11, 1953) stated that the patient was riddled with metastases and was rapidly going downhill. He died at home shortly thereafter, and permission for autopsy was not obtained.

Pathological Findings.—Gross: The specimen consisted of testicular tissue in several pieces and was obviously removed by subcapsular resection. They consisted of moderately firm brownish-tan tissue weighing 6 gm. The tubules could be stripped with ease in some portions.

Microscopic Findings: The testes were largely replaced by a well-differentiated glandular carcinoma. The glands were closely packed and lined by low columnar cells with large rounded bizarre nuclei. In some areas no normal testicular tissue was seen; whereas in other regions atrophic spermatic tubules were present, and carcinomatous elements permeated between tubules. The tumor glands often contained a basophilic mucoid substance within lumina.

The diagnosis was carcinoma of testes from carcinoma of the prostate.

Case 2.—C. C., a 72-year-old Negro man, was first seen in St. Philip Hospital on April 13, 1945, complaining of dribbling and decrease in the caliber of his urinary stream for four months. Physical examination at this time revealed a hard nodule in the right lateral lobe of the prostate and a residual of 6 cc. of urine. It was felt that this man had carcinoma of the prostate, and he was given 1 mg. of diethylstilbestrol three times a day. His treatment was continued for six weeks, after which time the patient stated he had no trouble in passing his urine and that his stream was markedly improved. No pathologic study of the prostate was made.

This patient was supposed to return for periodic check-ups in the Urology Outpatient Department, but he failed to do so and was not seen again until Nov. 18, 1952, at which time he entered the emergency room of the St. Philip Hospital in acute retention. He gave a history of having no trouble voiding until three months prior to admission, at which time he began to have burning pain in his bladder, and his urine was cloudy and purulent. He had nocturia (three or four times) with dribbling and difficulty in starting his stream during the past three weeks, and these symptoms became progressively worse until acute retention resulted. Positive physical findings on admission consisted of marked generalized arteriosclerosis, distended bladder relieved by catheter drainage, and an enormous prostate, which practically filled the rectum and which was felt to be fluctuant in the center and quite boggy. Laboratory work on admission revealed urine loaded with white blood cells. P. S. P. test revealed only 20% excretion in two hours, with good volume, and the blood urea nitrogen was 22 mg. per 100 cc. Despite a urinary output of over 1,500 cc. every 24 hours, the patient's blood urea nitrogen the first week in the hospital rose to 70 mg. per 100 cc. A cystotomy was done on Nov. 26, 1952. At this time a needle was stuck through the perineum, and 30 cc. of dark brownish material was obtained from the fluctuant area in the prostate. This was smeared and examined by Papanicolaou's method. Cells were found which were interpreted to be malignant (Class IV [See pathology report below]). The patient was placed on diethylstilbestrol 5 mg. a day, and forced fluids. By Dec. 19, 1952, the patient's B. U. N. was 14 mg. per 100 cc., and an orchidectomy was done on Dec. 19, in view of the fact that carcinoma cells had been found on fluid aspirated from the region of the prostate. After the orchidectomy, the patient did well. He was markedly improved, having gained weight; his appetite was better, and all blood chemistry studies at the time of discharge were within normal limits. While in the hospital, x-rays of the chest, pelvis, and spine were negative for metastatic disease; the acid and alkaline phosphatase values were within normal limits.

The patient was brought into the hospital again in June of 1953, and a resectoscope was passed. Approximately 5 gm. of tissue was removed, which cut similar to carcinoma, with no bleeding. There was no definite evidence of tumor histologically, though considerable estrogen effect was found.

Pathological Findings.—Gross (S-52 7883): Aspirated material from prostate. The specimen consisted of approximately 15 cc. of opaque dark red fluid with a small amount of soft whitish sediment, and the second consisted of approximately 20 cc. of similar fluid.

Microscopic Findings: There were masses of erythrocytes and moderate numbers of lymphocytes and polymorphonuclear leucocytes. Most conspicuous were numerous clusters of fairly large cells with overlapping, variously-shaped nuclei. The nuclear membranes were sharp, and the chromatin particles were unevenly distributed. A cell block showed irregular glandular structures made up of fused, variously shaped glands, each of which were lined by several layers of low columnar epithelial cells. Their structures resembled prostatic acini but were atypical. There was secondary acinar formation and a tendency to form small papillae (Fig. 1).

The diagnosis was fluid from prostatic cyst, positive for tumor (Papanicolaou Class IV).

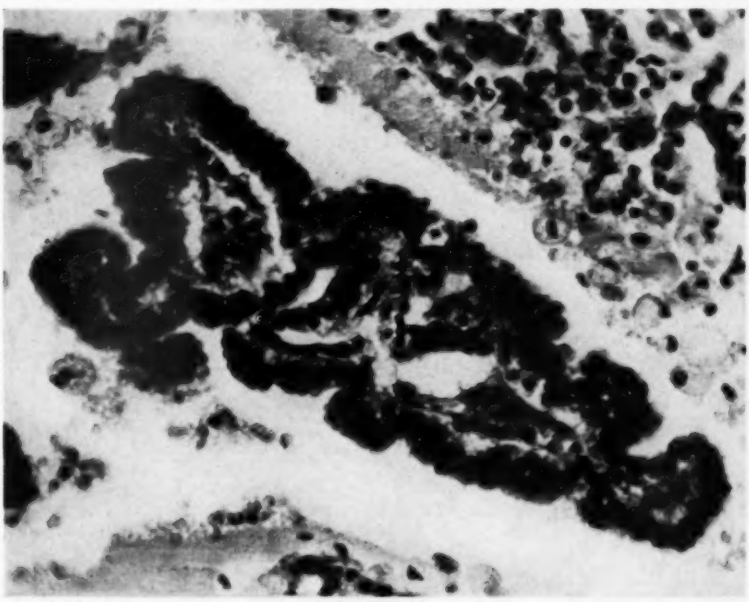


Fig. 1 (Case 7).—Material aspirated from enlarged and cystic prostate; × 220.

Gross (S-52 8258): The specimen consisted of testes and epididymides. The testes were equal in size and measured approximately 5 by 2 by 1 cm. On cut section one testis disclosed distinct yellowish infiltrations.

Microscopic Findings: Section through the testis containing the yellowish infiltrations revealed a neoplasm of an unusual type. The testis was extremely atrophic, and no normal spermatic tubules could be identified. Most showed complete hyalinization and only remnants of Sertoli cells. Some of them were lined by neoplastic cells arranged in columnar fashion and containing rounded bizarre nuclei with prominent nucleoli. The nuclei were deeply staining and stood out well among the lighter-staining Sertoli cells (Fig. 2). In almost every instance a well-defined lumen could be seen, and occasionally there were papillary infoldings (Fig. 2). In only one area could neoplastic glands be identified infiltrating the interstitium of the testis (Fig. 3). In this region the glands were irregular, and the lumen was filled with

basophilic substance identified as mucin with a mucicarmine stain. Mucicarminophilic material could also be seen within epithelial tumor cells lining the spermatic tubules.

The diagnosis was carcinoma of testis, unilateral, following carcinoma of the prostate.

CASE 3.*—J. D., a Negro man, age 54 years, was first admitted to the Johns Hopkins Hospital on Oct. 30, 1945, with the complaint of a dull aching pain in the back of 18 months' duration. A history of abnormal urinary function or signs could not be elicited. During the past 10 months he had lost 20 lb. (9.1 kg.). Physical examination revealed a slender well-developed Negro man in great pain. Pressure on the spinous processes elicited tenderness, especially involving the lower thoracic and upper lumbar vertebrae. In addition, there was distinct manubrial tenderness. The prostate was considered to be one and one-half times the

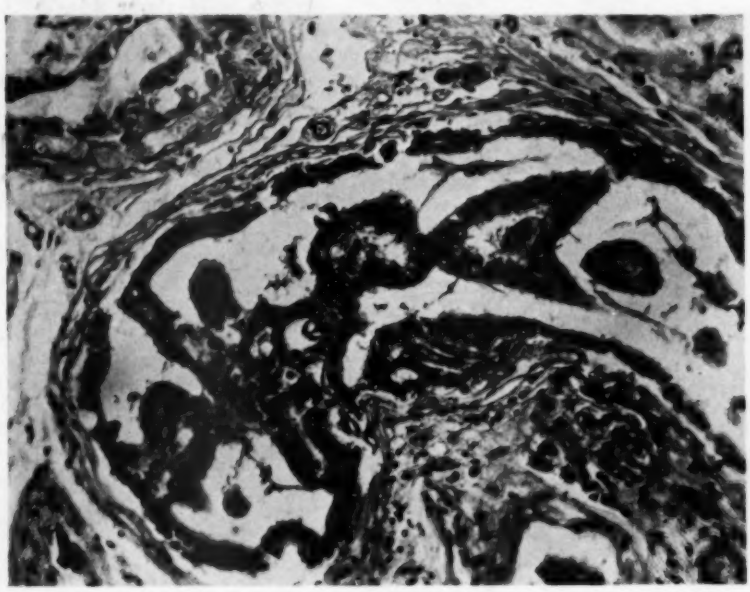


Fig. 2 (Case 2).—Metastatic tumor of testis. Note that the neoplasm is confined to the spermatic tubules. The uninvolved tubules are atrophic; × 200.

normal size. The enlargement was mainly on the right side, where the organ was particularly hard and fixed, with involvement of the seminal vesicle. Laboratory studies showed an increased sedimentation rate and elevated alkaline and acid phosphatase. Repeated roentgenographic examinations of the entire skeleton failed to reveal any lesions. The clinical impression was carcinoma of the prostate, with metastases to the vertebrae. The patient was placed on 1 mg. of diethylstilbestrol per diem and was discharged. The entire prostate became smaller, accompanied by softening of the right lobe.

The second admission was Nov. 7, 1947, at which time a right hydrocelectomy was performed. X-ray examination on this admission demonstrated multiple areas of increased density throughout the pelvis and upper femora.

The last admission was Nov. 29, 1948, three years after the first admission. The patient had been free of pain until four months previous to the last admission. The pain was severe and involved the low back, right hip, right shoulder, and both knees. Examination of the

* This case is being published in greater detail.[6]

prostate revealed third degree induration throughout the entire gland, with involvement of the seminal vesicles and fixation to the lateral walls of the pelvis. A bilateral orchidectomy for the relief of pain was performed.

Pathological Examination of the Testes (S. P. 48-4486).—Gross: The left testicle with its epididymis weighed 26 gm. The right testicle and epididymis weighed 28 gm. On section, the parenchyma of the testicular tissue was firm and translucent, with a rubbery consistency. The tubules did not "string out." No lesions were seen on examination of either epididymis.

Microscopic Findings: Both testes and epididymides showed identical features. The seminiferous tubules were uniformly markedly atrophic, and ramifying between them neoplastic strands of cells were plentiful. Here and there distinct gland formations of malignant cells were evident (Fig. 4). There was considerable collageniza-

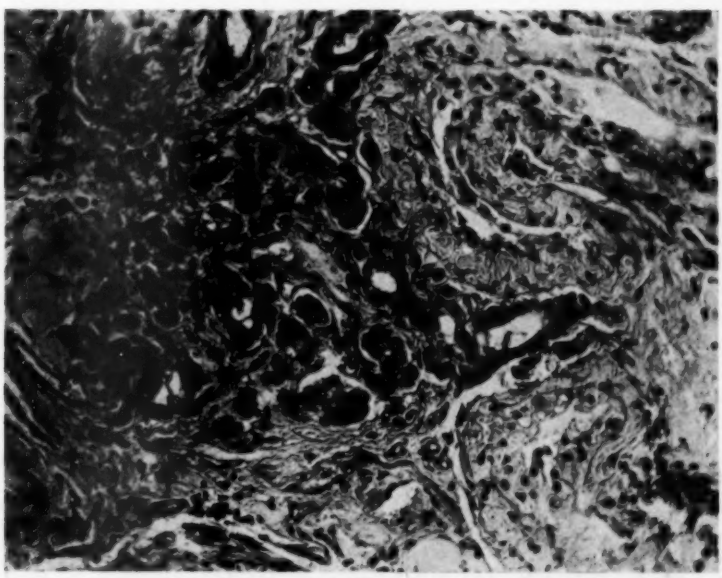


Fig. 3 (Case 2).—Invasion of tumor within interstitium of testis; × 220.

tion of the interstitial stroma. The nuclei of the malignant cells were extremely hyperchromatic, and it was difficult to differentiate nucleoli from atypical mitoses. There was very little vascularity noted, and Leydig cells were difficult to identify. In none of the sections was there invasion of the capsule. Examination of the epididymis failed to reveal any pathological change.

The diagnosis was carcinoma of the testes following carcinoma of the prostate.

After the operation the patient was discharged considerably improved. Several weeks after returning home, pain in the back and extremities recurred, and the patient went progressively downhill and died March 31, 1949, three years and four months after the first admission. The total duration of the illness was four years and 10 months. Permission for autopsy was obtained.

Autopsy Findings (No. 21703).—The appearance was that of an extremely emaciated, fairly well-developed Negro man with a physical presentation younger than the stated age of 54 years. There was generalized superficial lymphadenopathy. A palpable mass of lymph nodes was demonstrated in the left supraclavicular region. The greater omentum showed minimal fat content, and the lymph nodes of the mesentery were enlarged. Examination of the thoracic

contents revealed the presence of prominent mediastinal lymph nodes infiltrated with neoplastic tissue. Many of these measured 1.5 cm. in greater diameter. On section, the lungs were studded with tiny tumor nodules located subpleurally. The liver presented the "nutmeg" appearance of chronic venous congestion. The prostate was of normal size and indurated throughout. No median bar was evident. Fine tiny nodules, hard in consistency, were demonstrated just beneath the capsule of the lateral aspects of the organ. The bases of the seminal vesicles were firm and infiltrated with tumor tissue. Malignant involvement of iliac, inguinal, para-aortic, mediastinal, supraclavicular, and axillary lymph nodes was evident. A post-mortem radiological survey disclosed a marked osteoblastic reaction involving the entire vertebral column, pelvis, ribs, sternum, and long bones. No lesions were seen in the brain.

On histological examination, tumor was found in the prostate, seminal vesicles, lymph nodes, liver, lungs, and bones and was similar in all organs involved. The

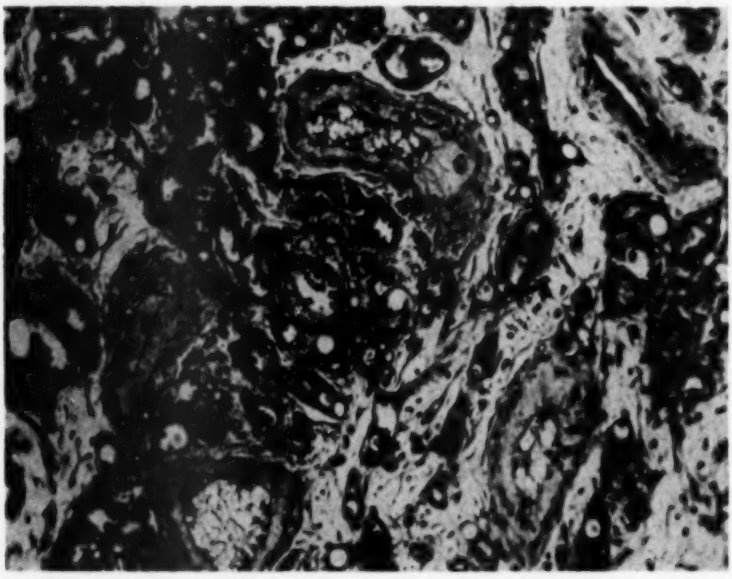


Fig. 4 (Case 3).—Metastatic tumor of testis. Note that the tumor is in the interstitium and that adjacent spermatic tubules are atrophic; × 200.

tumor in the prostate showed small acini, strands, and alveolar formations. Perineural lymphatic invasion was seen here and there. The prostate also revealed a few hyperplastic glands but for the most part was completely displaced by infiltrating tumor cells embedded in abundant collagenous tissue. Squamous metaplasia of the ducts was moderate in amount and was probably due to the effect of diethylstilbestrol therapy.

The anatomical diagnosis was carcinoma of the prostate gland, with metastases to the seminal vesicles, regional and distant lymph nodes, liver, lungs, and bones.

COMMENT

In the Table can be found the pertinent data concerning the previous cases reported and the present cases. Two cases did not have histologic confirmation of prostatic cancer, namely Baird and Hare's case [3] and our Case 2. It is indeed

Summary of Findings in Cases of Carcinoma of the Testes Metastatic from Carcinoma of the Prostate

Author	Wt. of Testis	Measurement of Testis	Gross Features of Testes	Side of Involvement	Other Metastases	Duration of Illness	Comments
Semans	Not given	"Normal in size"	Focal infiltration by a firm white substance	Right	Pelvis, lumbar vertebrae, presacral lymph nodes	6 mo.; death; autopsy	Carcinoma in interstitial tissue, vein, lymphatics; 1 arterial embolus?
Helfert and Pinck	Not given	Not given; subcapsular enucleation	Left testicle showed green-pea-sized nodule of firm grayish white tissue	Left	Skull, lumbar vertebrae, pelvic bones, femora	Over 4 yr.; alive at last follow-up	Tumor within interstitium
Baird and Hare	Not given	Not given	Left testicle showed grayish white nodule 10 by 7 mm. beneath tunica albuginea	Left	Ribs, pelvis, left femur	1 yr. 8 mo.; alive at last follow-up	Interstitial infiltration in testis and epididymis; no prostatic tissue ever examined microscopically
Bradham	Not given	5 by 4 by 3.5 cm.	Homogeneous, soft, bulging; substance friable; papillary-like tumor tissue present	Right	None at completion of study	1 yr. +; alive at last follow-up	Tumor tissue demonstrable in veins and tubules of testis, as well as in interstitium
Kay, Hennigar, and Hooper Case 1	6 gm. (only subcapsular tissue)	Unable to ascertain accurately	No distinctive features	Bilateral	D 12, L 1, iliac bones	6 yr.; death; no autopsy	Tumor only found in interstitium
Case 2	Not done	5 by 2 by 1 cm.	Yellowish infiltrations	Unilateral	None at completion of study	8 yr.; alive at last follow-up	Tumor principally within spermatic tubules; only 1 small area of invasion into interstitium
Case 3	Left, 26 gm.; right, 28 gm.; epididymides included in wt.	Not done	Diffuse changes characterized by opacity and loss of stringiness of tubules	Bilateral	Lymph nodes, liver, lungs, vertebral column, pelvis, ribs, sternum, long bones	4 yr. 10 mo.; death; autopsy	Tumor only within interstitium

unfortunate that patients with prostatic carcinoma all too frequently fail to have histologic verification of their tumors (this occurred also in Case 3 prior to the autopsy). Often the clinician decides that a good response to steroid hormone is bona fide evidence that a malignant tumor did actually exist in the prostate. This is not always true, since patients with benign enlargements of the prostate will sometimes show prostatic shrinkage and improvement of urinary signs after diethylstilbestrol therapy and castration.* In general, therefore, failure to secure an adequate biopsy of the prostate in suspected malignant tumors is to be deplored.

Despite the absence of positive histologic proof, Baird and Hare's case [8] seems convincing enough, since the clinical evidence is overwhelming. Our Case 2 may reasonably be questioned, since the testicular lesion might be considered a neoplasm of excretory ducts, for example, Sertoli cell tumor, or androblastoma. We believe that the positive mucin stain would most certainly exclude such a diagnosis. A comparison of Figures 1 and 2 shows that the cellular elements of the aspirated material from the prostate and the testicular lesion are sufficiently similar to be considered the same neoplasm. Since a number of prostatic carcinomas in our laboratory have shown mucin secretion, it seems logical to conclude that the prostate gland harbors the primary tumor and that the testicular growth is metastatic.

In no instance was a positive clinical diagnosis of prostatic metastases to the testis made. Only Case 3 showed any appreciable enlargement of the testes, although measurements of these organs is woefully lacking.

From the pathologic standpoint, the gross features can be quite helpful. Four cases showed focal infiltrations of obvious neoplastic tissue; two showed diffuse changes, and in only one were there no distinctive features.

The duration of illness is of interest, since the majority of patients had their disease for at least four years. Possibly the added length of the disease increases the chances for testicular metastases to develop.

Concerning the pathogenesis of secondary testicular tumors, we have one other route of spread to suggest in addition to those mentioned by Willis.[5] It is possible that prostatic tumor may descend by way of the ejaculatory ducts to ultimately reach the epididymis and testis. Should this type of dissemination occur, tumor would be found in the spermatic tubules. This was the principal state of affairs in our Case 2, and tumor tissue was demonstrable in some of the tubules in Bradham's case.[4]

Finally, it is believed that more cases of testicular metastases from a primary prostatic carcinoma exist than appear in the literature. The availability of testicular tissue for study resulting from therapeutic orchidectomy should make it mandatory that testes removed at operation for whatever cause be submitted to the pathology laboratory for histologic examination.

SUMMARY

The available literature concerning carcinoma of the testis following carcinoma of the prostate is reviewed.

Three new cases of metastatic carcinoma of the testis following a primary prostatic neoplasm are presented.

* References 7 and 8.

A plea is made to secure biopsies of suspected prostatic neoplasm whenever such biopsy is feasible and not contraindicated.

The importance of submitting all testicular tissue removed for whatever cause to the pathologist for histologic examination cannot be overemphasized.

REFERENCES

1. Semans, J. H.: Carcinoma of the Prostate with Metastasis in the Testis, J. Urol. **40**:524-529, 1938.

2. Helfert, I., and Pinck, B. D.: Secondary Carcinoma of the Testicle Following Carcinoma of the Prostate, J. Urol. **51**:635-640, 1944.

3. Baird, S. S., and Hare, D. M.: Metastasis of Prostatic Carcinoma to Testicle, J. Urol. **59**:1208-1211, 1948.

4. Bradham, A. C.: Prostatic Carcinoma with Metastasis to the Testicle, J. Urol. **66**:122-126, 1951.

5. Willis, R. A.: The Spread of Tumours in the Human Body, Ed. 2, London, Butterworth & Co., Ltd., 1952, pp. 287-289.

6. Hennigar, G. R., and Scardino, P. L.: Carcinoma of the Prostate with Metastases to the Testes, to be published.

7. Heckel, N. J.: Evaluation of Sex Hormones in the Treatment of Benign Prostatic Hypertrophy, Carcinoma of the Prostate and Other Diseases of the Genito-Urinary System, J. Clin. Endocrinol. **4**:166-172, 1944.

8. Huggins, C., and Stevens, R. A.: The Effect of Castration on Benign Hypertrophy of the Prostate in Man, J. Urol. **43**:705-714, 1940.

EFFECTS OF *DL*-ETHIONINE ON THE PANCREAS AND OTHER ORGANS

MARIO ALVIZOURI, M.D.
MEXICO, D. F., MEXICO
AND
SHIELDS WARREN, M.D.
BOSTON

PANCREATIC acinar necrosis * in the rat, followed by rapid regeneration,[3] is induced by DL-ethionine (alpha-amino-gamma-ethylmercaptobutyric acid), an analogue of methionine. It has been reported also to produce fatty metamorphosis † and fibrosis of the liver ‡ and renal [7] and adrenal lesions.[4]

These experiments were designed to observe the effects of acute and chronic treatment with DL-ethionine and to study acinar pancreatic regeneration. In the course of this study severe testicular lesions were for the first time observed.

MATERIAL AND METHODS

Acute Experiment.—Sixty Slonaker rats, male and female, weighing between 100 and 150 gm. were divided into two groups which received 0.5 and 1 mg. of DL-ethionine daily intraperitoneally for 18 days. The DL-ethionine was in 2.5% aqueous solution. Food and water were permitted ad libitum. Thirty-six rats were killed at intervals from 12 hours to 30 days after the treatment began. Twenty-four of these rats were on daily treatment for 5, 10, or 15 days and were killed 30 days after cessation of treatment.

Forty more rats were fed their usual food (Purina Laboratory Chow) with 0.5% of DL-ethionine added. Half of these rats in addition received 0.5% of pancreatic extract (Pancreatin, of Parke, Davis & Company) also mixed with the food. It was estimated that each of these rats received between 100 to 200 mg. of DL-ethionine daily. The rats were killed as above described.

Chronic Administration.—Sixty Wistar white rats of both male and female sexes, weighing about 150 gm. at the beginning of the experiment, were submitted to treatment with DL-ethionine for seven successive courses. The DL-ethionine was mixed with the food in a proportion of 0.5%. Water was permitted ad libitum. Each course lasted from 14 to 18 days and was followed by an interval lasting from 15 to 30 days. Two to four animals were killed at the end of each course to observe the pathologic effects of the drug, and the same number were killed at the completion of the resting period to study the stage of regeneration of the pancreas.

In all the groups complete autopsies were performed, and samples of all organs were fixed in Zenker's fluid and stained with hematoxylin and eosin. Livers were also stained with Masson's trichrome technique. The tissues of 60 rats of the acute experiment were frozen-dried, embedded in paraffin, and stained with toluidine blue (with and without ribonuclease digestion), phosphotungstic acid hematoxylin, Gomori's chrome alum hematoxylin, and the Feulgen stain.

From the Cancer Research Institute of the New England Deaconess Hospital and the Laboratory of Pathology, Harvard Cancer Commission, Boston.

* References 1 and 2.

† References 1 and 4.

‡ References 5 and 6.

RESULTS AND COMMENT

To avoid confusion, the results will be described by organ, in order of importance and frequency of lesion, as follows: pancreas, testis, liver and kidney.

Pancreas.—With acute treatment similar results were observed in the animals treated orally and parenterally. The group treated parenterally showed more consistent and slightly more marked lesions. A small group of rats that began the experiment receiving 1% of DL-ethionine mixed with the food showed a high index of mortality, for which reason the proportion of DL-ethionine was reduced to 0.5%. No protection, either clinical or pathologic, was afforded by the addition of pancreatic extract.

The fundamental pathologic process found consisted of a progressive atrophy and necrosis of acinar cells. Within 10 to 14 days of treatment, about 95% of the rats, regardless of sex, showed marked atrophy of the acinar portion of the pancreas. Only a few scattered acinar cells were identified by the presence of relatively abundant cytoplasm and a few remaining zymogen granules. In our material fat necrosis has never been present.

The earliest lesion noticed after the administration of DL-ethionine was a marked reduction in the amount of chromidial substance, which was studied with toluidine blue. Further identification of the chromidial substance was made by the use of ribonuclease, which produced its digestion. The depletion of the chromidial substance began within the first 24 hours, increasing rapidly with the treatment and returning to normal after the treatment ceased. There was never complete absence of the chromidial substance, unless the acinar cells were completely atrophic. This is in disagreement with the data reported by other workers who have regarded the total disappearance of the chromidial substance as a constant and early finding. This difference is undoubtedly due to the use of different histological techniques.

The zymogen granules, studied by means of the phosphotungstic acid hematoxylin stain, remained present in the cytoplasm as long as the acinar cells were still identifiable. Zymogen granules were absent only in far-advanced lesions, when the acinar cells were completely atrophic and could not be differentiated from the duct cells or the stroma.

Fifteen days after the cessation of administration of the drug the pancreas was completely regenerated. The study of the chromidial substance and zymogen granules during this period indicated that the remaining acinar cells were the source of acinar regeneration. Occasional mitotic figures began to appear about the seventh day of treatment in isolated foci of acinar cells and were abundant after the cessation of administration of the drug. No acinar regeneration could be seen originating from the pancreatic ducts, and mitoses were rare there.

After each course of chronic treatment the acinar portion of the pancreas showed marked atrophy. In the most advanced lesions only about 5% of the acinar tissue survived. At this stage the pancreas can best be described as a skeleton of branching ducts with occasional acinar cells and supported by adipose tissue. The ducts and acinar cells tended to form isolated nodules. Langerhans' islets were scattered in the fatty tissue, associated always with a small pancreatic duct and less often with acinar cells. This picture was essentially the same at the end of each course, with only variations of degree.

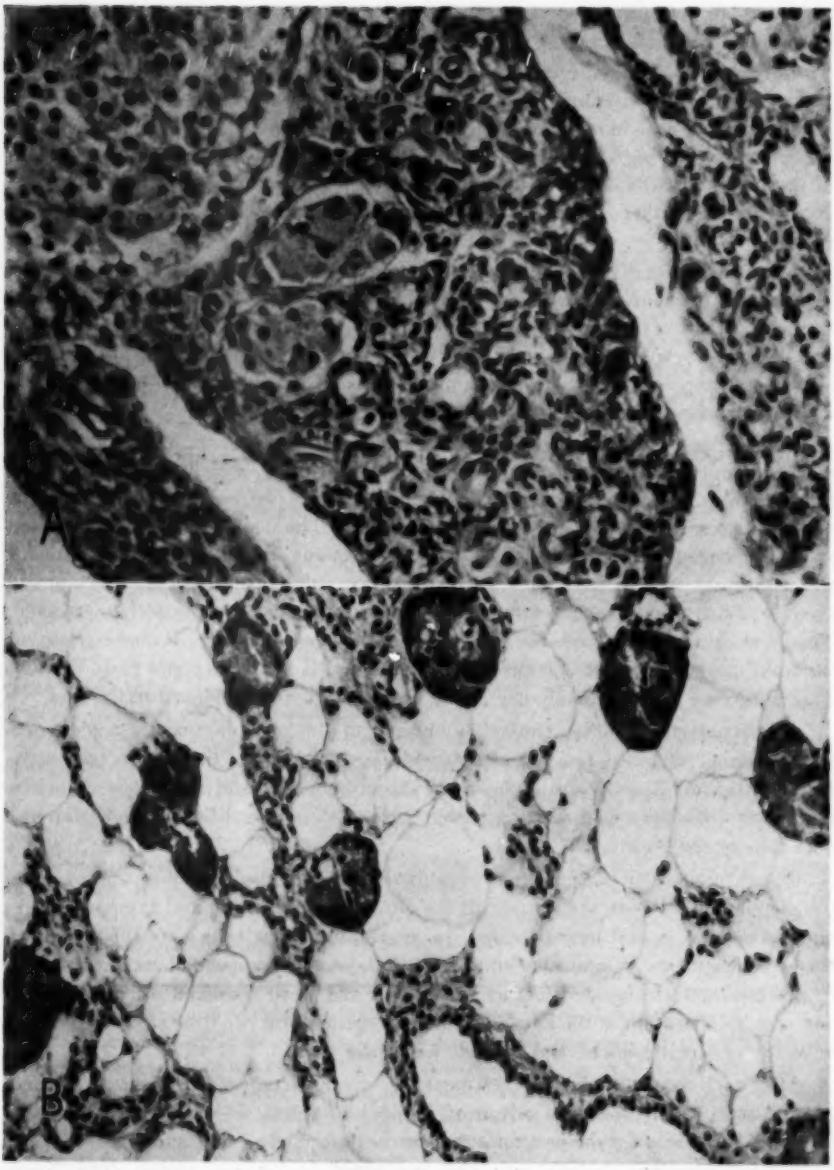


Fig. 1.—*A*, the pancreas after treatment with DL-ethionine for 15 days; × 400. *B*, atrophy of the pancreas after three courses of DL-ethionine; × 250.

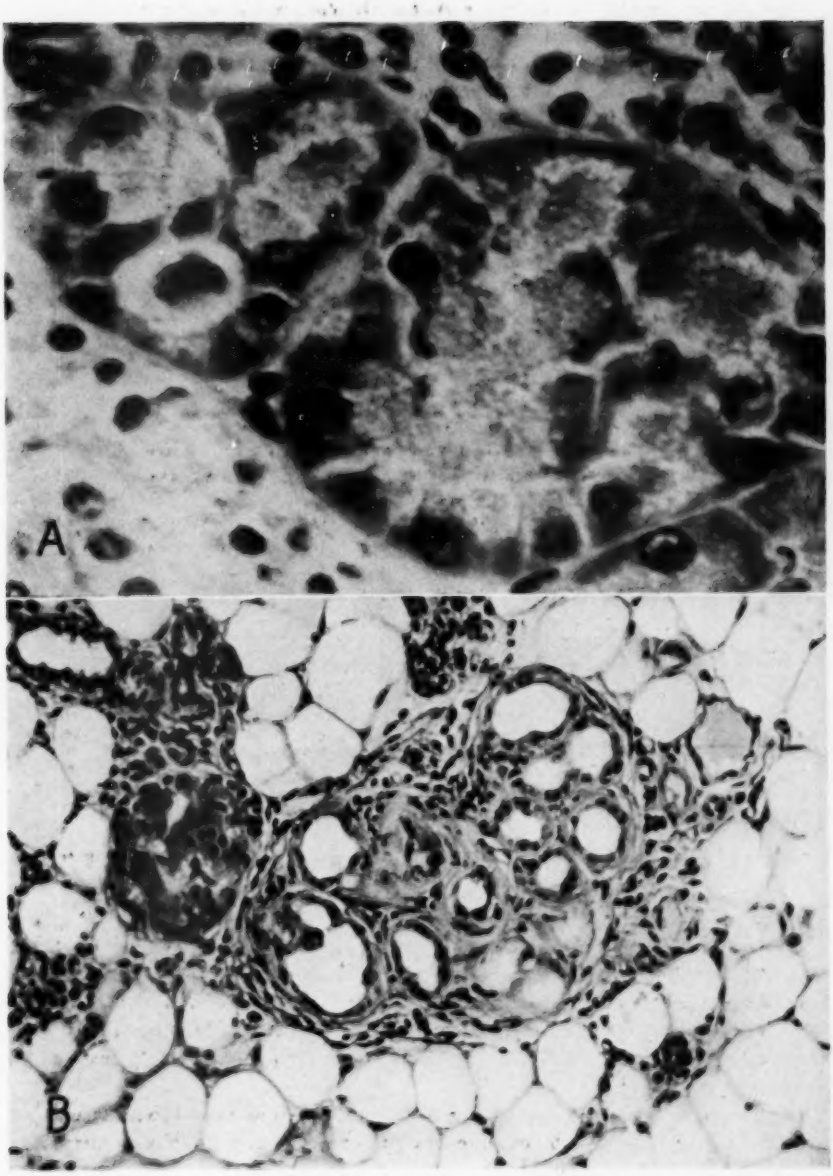


Fig. 2.—*A*, pancreatic atrophy and duct proliferation during fourth course of DL-ethionine treatment; × 250. *B*, regenerating acinar cells during recovery from DL-ethionine treatment; × 1,000.

During the periods of rest the pancreas showed partial or complete regeneration, depending on the time allowed to rest. In general, regeneration after the second and subsequent courses was not so rapid as after a single course. With a month period of rest the pancreas showed complete restitution to the normal pattern, even after the seventh course. With rest of 15 days the pancreas showed regeneration, represented by nodules of acinar cells and ducts scattered in the fat tissue. Foci of regeneration occurred always adjacent to semiatrophic acinar cells. However, ducts and centroacinar cells could not be completely excluded from taking part in the process of acinar regeneration. Proliferation of ducts was, in general, moderate. Only in a few instances had ducts proliferated enough to form a nodule.

Blood sugar determination by the micromethod of Folin [8] was done in 12 rats during the first five courses. This showed normal blood sugar levels (Table 1). In correlation, the islets of Langerhans in both the acute and the chronic experiments showed no morphologic changes, with the exception of a few animals, which after

TABLE 1.—*Blood Sugar Values* *

		1st Course		2d Course		3d Course		4th Course		5th Course	
Rat No.	Before Experiment	Last Day of Treatment	Last Day of Rest	Last Day of Treatment	Last Day of Rest	Last Day of Treatment	Last Day of Rest	Last Day of Treatment	Last Day of Rest	Last Day of Treatment	Last Day of Rest
1	98	70	77	86	...	95	...	87	100	..	...
2	92	91	115	95	105	97	109	..	112	84	105
3	82	..	...	128	110	105	100	98	105	96	...
4	77	77	104	105	102	98	115	..	98	104	...
5	92	82	126	..	98	103	...	..	105	..	...
6	75	82	97	85	95	97	...	89	95	..	...
7	98	88	...	90	98	85	110	105	...	91	...
8	100	76	92	90	...	105	120	115	...	76	110
9	73	83	98	89	102	118	105	110	99	98	105
10	80	70	98	86	100	..	115	128	...	98	120
11	80	76	104	98	90	110	106	130	...	94	106
12	..	77	100	100	...	95	...	105	115	98	105

* Milligrams per 100 cc. in DL-ethionine-treated animals.

the second course of treatment showed slight vacuolization of the cells in a few of the islets.

Testis.—With a daily intraperitoneal dose of 0.5 mg. of DL-ethionine per gram of body weight, the testis showed minimal pathologic effects about the seventh day of treatment, becoming marked by the 10th day. With the higher dose (1 mg. per gram of body weight) the lesions began to appear about the fourth day and were more marked by the seventh day. The degree of degeneration advanced rapidly with the treatment, and by the 16th day the seminiferous epithelium was represented by Sertoli cells only.

During the first 7 to 14 days the response of the seminiferous tubules was very irregular; while in some tubules degeneration was advanced, in others spermatogenesis was apparently normal.

Relatively more marked lesions were observed in the animals that, injected for periods of 5, 10, or 15 days, were killed 30 days after the last injection. The effects on this group can be observed in Table 2.

With repeated courses of DL-ethionine administered orally, the testicular lesions were similar but less marked. It was observed that even in animals that received

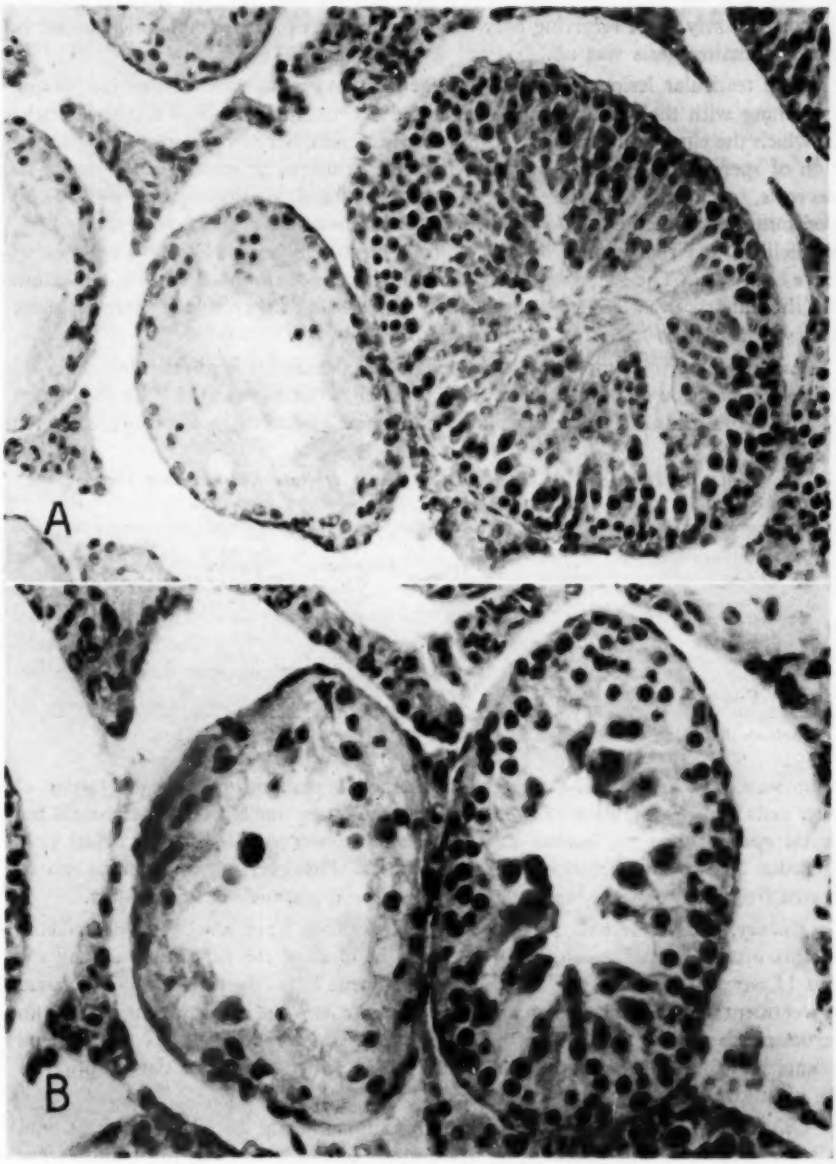


Fig. 3.—*A*, testicular degeneration after treatment with DL-ethionine. Atrophy of most tubules. One tubule with intact spermatocytes and spermatogonia but degenerated spermatids; × 250. *B*, testicular degeneration after DL-ethionine treatment. Note one tubule with spermatogonia and spermatocytes, the other with almost complete degeneration; × 400

several courses of DL-ethionine the testis still showed foci of spermatogenesis, although rarely those receiving only one or two courses showed much degeneration, and spermatogenesis was nil.

The testicular lesions consisted of degeneration of the seminiferous epithelium, beginning with the spermatids. Degenerated spermatids contained eccentric nuclei in which the chromatin was displaced toward the periphery by vacuoles. Agglutination of spermatids, forming large polynucleated clumps, as seen in rapid testicular necrosis, occurred only rarely. Spermatocytes and spermatogonia were more resistant and were the last to disappear. In the most advanced lesions the tubular epithelium was represented by Sertoli cells only. No lesions of the Leydig cells have been seen. The sequence of events of the lesions of the seminiferous epithelium is different from that seen in x-irradiation of the testis, in which the spermatogonia and spermatocytes [9] are first to be affected.

Liver.—Fatty metamorphosis,§ cholangiolitis, proliferation of reticuloendothelial cells, degeneration of hepatic cells, and fibrosis have been reported.‖ In the course of this experiment fatty metamorphosis and acute and subacute cholangiolitis have

TABLE 2.—*Effects of* DL-*Ethionine on Testes of Rats Killed Thirty Days After End of Treatment*

No. of Animals	Days Treated	Daily Dose, Mg.	Degree of Tubular Degeneration	No. of Tubules with Spermatogenesis
4	5	0.5	++	Few
4	5	1	+++	Few
4	10	0.5	+++	Exceptional
4	10	1	++++	None
4	15	0.5	++++	None
4*	15	1	....	.....

* Animals died before treatment was completed.

been seen, but no fibrosis or reticuloendothelial proliferation. Degeneration of liver cells and proliferation of the bile ducts, the latter more prominent around the portal spaces, were the lesions most consistently observed. In one case duct proliferation led to the formation of a cholangioma. However, no conclusions can be drawn from a single case, and more work has been planned on this subject.

Kidney.—Renal lesions produced by DL-ethionine have also been described.¶ In this material renal lesions were observed in most of the rats killed during the first 15 days of treatment but were absent in animals killed during the second course of treatment or later. The lesion appears reversible, and the renal tubular epithelium becomes resistant or adapted to the action of DL-ethionine. The peak of the renal lesions was found between the 6th and 12th day and consisted of degeneration of the proximal convoluted tubule.

Other Organs.—Owing to the histological similarity between pancreas and salivary glands, it was expected to find lesions in the latter, but the results were negative. Because of the changes in the chromidial substance of the pancreas, special attention was directed toward changes in the basophilic substance or Nissl's

§ References 1 and 4.

‖ References 5 and 6.

¶ References 4 and 7.

substance of the neurons, which was always found normal. No lesions were found in other organs, among which special attention was paid to adrenals, ovaries, and bone marrow. Adrenal hemorrhages [4] have been reported, but our material failed to show such a lesion.

Marked depletion of fat was usual, resembling in some instances the gross picture of the so-called serous atrophy of fat. This was more evident in the subcutaneous, omental, perirenal, and periadrenal fat. This depletion of fat correlated closely with the marked loss of weight.

DL-Ethionine was tried in the treatment of two different transplantable malignant tumors in rats and mice. For this purpose tumor EO-771 (mammary gland carcinoma of mice) was transplanted subcutaneously to 20 mice (C-57 black line). Also, a Walker tumor was transplanted subcutaneously to 20 Slonaker white rats. Half of the mice and half of the rats were given 0.5% DL-ethionine mixed with the food as soon as the tumor became palpable. The other half of the animals were utilized as controls, receiving no treatment. No clinical or histological difference was noticed between the tumors in the treated and the untreated animals.

SUMMARY

Rats treated with one or several courses of DL-ethionine showed pancreatic acinar atrophy and necrosis, followed consistently by regeneration. Regeneration of pancreatic acinar cells was traced to remaining semiatrophic acinar cells, whereas the ducts take no or minimal part in the process. Different degrees of testicular degeneration were observed with parenteral and oral administration of DL-ethionine. Hepatic and renal lesions were also produced by DL-ethionine. This substance failed to produce any effects on Walker tumor in rats and tumor EO-771 in mice.

Avida Niños Heroes 150 (Dr. Alvizouri)

REFERENCES

1. Goldberg, R. C.; Chaikoff, I. L., and Dodge, A. H.: Destruction of Pancreatic Acinar Tissue by DL-Ethionine, Proc. Soc. Exper. Biol. & Med. **74**:869, 1950.

2. Farber, E., and Popper, H.: Production of Acute Pancreatitis with Ethionine and Its Prevention by Methionine, Proc. Soc. Exper. Biol. & Med. **74**:838, 1950.

3. Fitzgerald, P. J., and Alvizouri, M.: Rapid Restitution of the Rat Pancreas Following Acinar Cell Necrosis Subsequent to Ethionine, Nature, London **170**:929, 1952.

4. Farber, E.; Simpson, M. V., and Tarver, H.: Studies on Ethionine: Interference with Lipide Metabolism, J. Biol. Chem. **182**:91, 1950.

5. Koch-Weser, D., and Popper, H.: Hepatic Fibrosis Produced by Chronic Ethionine Feeding, Proc. Soc. Exper. Biol. & Med. **79**:34, 1952.

6. Popper, H.; de la Huerga, J., and Koch-Weser, D.: Hepatic and Pancreatic Changes Produced in Rats by Ethionine and Their Relation to Human Lesions, Scientific Proceedings, Am. J. Path. **28**:518, 1952.

7. Wachstein, M., and Meisel, E.: Nephrotic Action of DL-Ethionine, Proc. Soc. Exper. Biol. & Med. **77**:648, 1951.

8. Folin, O.: The Micro Method for the Determination of Blood Sugar, New England J. Med. **206**:727, 1932.

9. Fogg, L. C., and Cowing, R. F.: The Changes in Cell Morphology and Histochemistry of the Testis Following Irradiation and Their Relation to Other Induced Testicular Changes: Quantitative Random Sampling of Germinal Cells at Intervals Following Direct Irradiation, Cancer Res. **11**:23, 1951.

MYELOMALACIA AND MULTIPLE CAVITATIONS OF SPINAL CORD SECONDARY TO ADHESIVE ARACHNOIDITIS

An Experimental Study

ROBERT L. McLAURIN, M.D.
CINCINNATI
ORVILLE T. BAILEY, M.D.
INDIANAPOLIS
PETER H. SCHURR, M.B.
LONDON, ENGLAND
AND
FRANC D. INGRAHAM, M.D.
BOSTON

THE PURPOSE of this report is to describe an experimental method for producing chronic adhesive arachnoiditis and to trace the pattern of spinal cord reaction resulting from this condition. The reaction eventually leads to cavitation of the spinal cord. Although such cavitations have been previously produced experimentally by other methods, the conditions of the present study resemble those of clinical arachnoiditis more closely than in the earlier investigations. Moreover, this work offers evidence from a new approach in regard to current theories of cavitation in the spinal cord resulting from vascular occlusions and from adhesive arachnoiditis, both experimental and clinical.

METHOD

During the course of experimental work on hydrocephalus, the injection of kaolin into the cisterna magna of laboratory animals was found to produce an adhesive arachnoiditis. Forty-five mongrel dogs were used for the present study. A suspension of 250 to 500 mg. of kaolin in 2 to 4 cc. of cerebrospinal fluid was injected into 26 of the dogs while the animals were under thiopental (Pentothal) sodium or pentobarbital (Nembutal) anesthesia. Thirteen dogs received cisternal injections of 3 to 6 cc. of ethyl iodophenylundecylate (Pantopaque). The present study is confined to the changes in the spinal cords of these animals.

The animals were killed or died of other causes from nine days to four and one-half months after the arachnoiditis was induced. In several of the animals, India ink was injected directly into the fourth ventricle a few hours before death in order to determine whether there was communication between the ventricular system and the cavitations in the spinal cord. With a little practice it was possible to insert a needle into the fourth ventricle by the suboccipital route without danger to the animal. This procedure was facilitated by hydrocephalus, which was present in all the dogs of the series.

Mr. Schurr is an Eli Lilly Traveling Fellow, Medical Research Council of Great Britain.

This study was supported in part by Grant U. S. P. H. S. B157 of the National Institute of Neurological Diseases and Blindness.

Dr. McLaurin's present address is The Division of Neurosurgery, University of Cincinnati College of Medicine, Cincinnati General Hospital.

From the Neurosurgical Service of The Children's Medical Center, Boston, and the Department of Neurology, Indiana University School of Medicine, Indianapolis.

The brains and spinal cords were removed at necropsy and fixed in 4% formaldehyde (10% formalin). Hematoxylin and eosin were used as the routine stain, supplemented by Mallory's phosphotungstic acid hematoxylin and Scharlach R when indicated.

CLINICAL OBSERVATIONS

A characteristic sequence of observable neurologic defects accompanied the development of myelomalacia. The first abnormality noted was unsteadiness of gait, with a tendency to walk or stand on a wide base. This corresponded to early involvement of the posterior columns and horns by the degenerative process. The unsteadiness progressed until the animal was unable to move freely from fear of falling. At this time there was apparent disregard for the relative positions of the limbs, as evidenced by the lack of effort to resume a normal posture after the limbs had been moved into an awkward position (for example, crossed or spread widely apart) by the examiner. This was also interpreted as the result of posterior column involvement. Still later stages of the degeneration included the motor tracts, as shown by spasticity and scissors position of the limbs. The animal was then unable to stand. In one animal a diminution of pain appreciation was demonstrated by the subcutaneous injection of hypertonic saline solution into the foreleg. Signs similar to those described were observed by Dixon and Heller [1] in animals which received cisternal injections of kaolin. The neurologic changes were attributed to hydrocephalus; however, the spinal cords of these animals were not examined.

PATHOLOGIC FINDINGS

When the brains and spinal cords of these animals were studied after removal and fixation in 4% formaldehyde, it was found that injected kaolin had become dispersed through the subarachnoid spaces at the base of the brain, posterior fossa and cervicothoracic spinal cord. The resulting arachnoiditis led to communicating hydrocephalus. This was also found in the 13 dogs receiving ethyl iodophenylundecylate.

In the present study, attention was focused on the myelomalacia and cavitation of the spinal cord. The exact incidence of these changes could not be stated with certainty, since detailed examination of the spinal cords were not made in a few of the earlier animals of the series. However, some degree of macroscopic cavitation was encountered in 25 dogs, more than half of those from which the spinal cord was removed. In the animals receiving injections of India ink into the fourth ventricle before death, the material could be seen in the central canal and the cavitations, establishing that communication existed in these structures.

The earliest changes visible grossly in the spinal cords were necrosis and softening of the posterior portion at the junction of gray and white matter. This was seen in one animal only nine days after the cisternal injection of kaolin. As the process advanced, slit-like defects were found in the posterior half of the spinal cord, usually in relation to the posterior horns (Fig. 1). The gray matter of the posterior columns was necrotic (Fig. 2) and was easily dislodged when the cord was sectioned after fixation. The central canal was often dilated at this time (four to five weeks). The final stage was that of cavitation. Several small cavities were present in the posterior half of the cords of animals killed at four to six weeks; in those surviving for longer periods the cavities became confluent and often com-

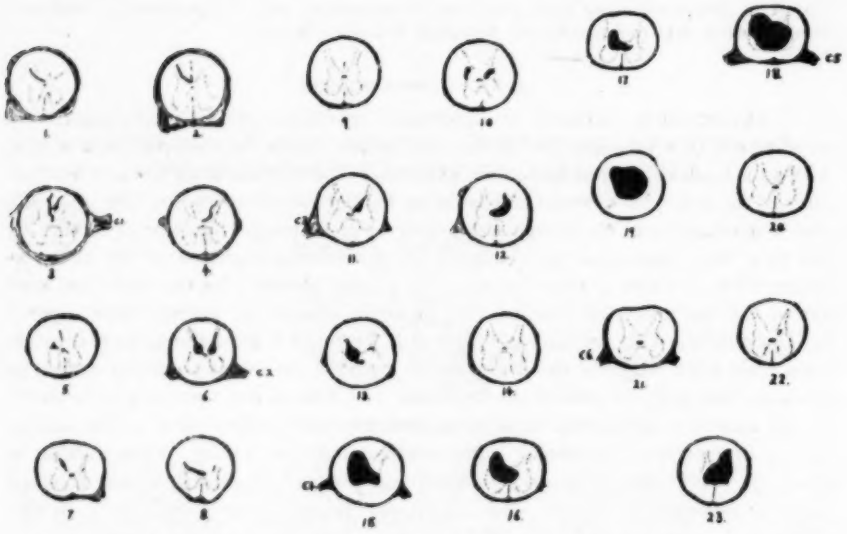


Fig. 1.—Sketches of successive blocks of the cervical spinal cord in Dog N-178-52. This animal was killed two months after the injection of kaolin into the cisterna magna. Note the slit-like defects and cavitations separated by intact spinal cord tissue.

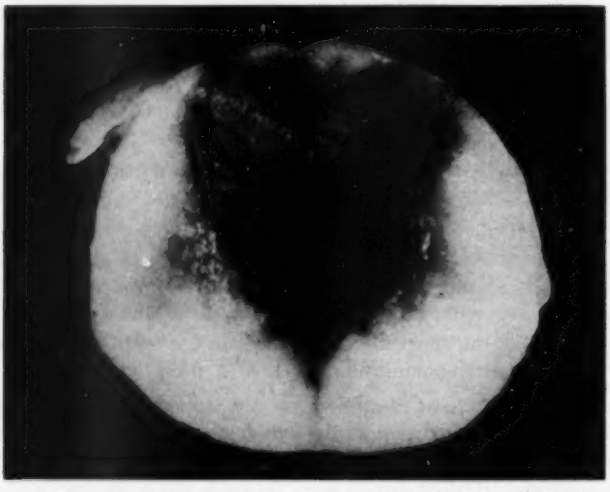


Fig. 2.—Gross photograph of a cross section of the cervical spinal cord in Dog N-164-52 ten weeks after the injection of ethyl iodophenylundecylate into the cisterna magna. The posterior portions of the spinal cord are necrotic. The cavitation continuous with the central canal is directly related to the area of necrosis. The histologic appearance is shown in Figure 5

municated with the central canal. Eventually a large syrinx developed, without visible evidence of a central canal. The largest of these were at least half the width of the spinal cord in diameter and involved primarily the posterior portion.

The process of degeneration and cavitation was most marked in the cervical region (Fig. 1) but occasionally extended well into the thoracic portion of the cord. The early degenerative process was patchy and irregular in distribution, without macroscopic continuity between successive degenerative areas. The early phase was demonstrated in several dogs during life by the injection of ethyl iodophenylundecylate into the fourth ventricle. The contrast medium outlined the thread-like central canal from which nummular projections extended posteriorly at irregular intervals (Fig. 3).

On microscopic examination there was an adhesive arachnoiditis completely surrounding the spinal cord in all animals. When kaolin was the material injected, there was a nearly solid ring of dense collagenous tissue extending from the pia to the dura and blending with the fibrous structure of the latter (Fig. 4). None of the collagenous tissue extended into the substance of the spinal cord, the pial margin being a sharp delimiting point. The vessels of the subarachnoid space were embedded in the fibrous tissue and appeared to be constricted by it. Throughout the collagenous tissue there were groups and masses of phagocytic cells filled with kaolin (Fig. 4). In a few areas there were larger unphagocytosed masses of kaolin surrounded by phagocytes and proliferating connective tissue, indicating that the reaction to kaolin was progressive and characterized by continuing phagocytosis and activity on the part of the fibrous tissue. Inflammatory cellular infiltration was minimal, being limited to a few groups of lymphocytes at widely separated intervals.

In the animals receiving ethyl iodophenylundecylate, the reaction in the spinal meninges was quantitatively similar, but the ethyl iodophenylundecylate was not visible in the sections.

The location and extent of the multiple outpouchings of the central canal in a representative dog are shown in Figure 1. Histologic sections taken at intervals between outpouchings showed the central canal to be normal or slightly dilated and lined by intact ependyma. In such sections the only abnormal feature was the adhesive arachnoiditis.

The changes leading to cavitations began abruptly. No special anatomic arrangement seemed to be involved, and no consistent pattern was found from animal to animal, other than that the cavitations were maximal in the cervical spinal cord, with less frequent involvement of the thoracic portions and none at lower levels.

When cavitations appeared, they usually extended so as to produce necrosis and rupture of the ependyma. The ependymal cells were preserved in those portions of the spinal cord not involved in the cavity but ceased abruptly at its margin and were wholly lacking in the cavity itself. The walls of the cavities were covered by necrotic tissue, beneath which were the attenuated and somewhat fragmented posterior tracts and fibers. Very little astrocytic reaction was seen; in most dogs, it was impossible to state with certainty that there was any (Fig. 5). About the cavities there was a mild increase in microglia, some of which contained phagocytosed material. Though blood vessels persisted on or near the lining of the cavitations, there was no evidence of hemorrhage or thrombosis. When India ink had been injected into the fourth ventricle before death, these vessels were surrounded by phagocytes filled with the material (Fig. 5).

Fig. 3.—Roentgenogram of Dog N-161-52 six weeks after the cisternal injection of kaolin. The contrast medium, which had been injected into the fourth ventricle, fills the central canal of the spinal cord and demonstrates nummular projections extending posteriorly at irregular intervals. The temporal horns of the lateral ventricles are also demonstrated.

Fig. 4.—Photomicrograph of the meninges in the cervical region of Dog N-106-52. This animal was killed four and one-half months after injection of kaolin into the cisterna magna. The anterior spinal artery and its branches are embedded in the fibrous tissue formed in reaction to kaolin. The kaolin is seen in the photograph as very dark masses in the meningeal region. The reaction stops abruptly at the pia. Hematoxylin and eosin stain; × 110.

From the histologic material, the impression was gained that ischemia led to necrosis of the spinal cord, which extended to include the ependyma of the central canal posteriorly. This provided free access of cerebrospinal fluid into the area of necrosis through the defect in the ependyma. Efforts to correlate severity of the meningeal reaction in particular segments with the formation of a particular cavitation were inconclusive.

The anterior horns and tracts were free from necrosis or other change and showed very little distortion, even in regions where the cavitations were large.

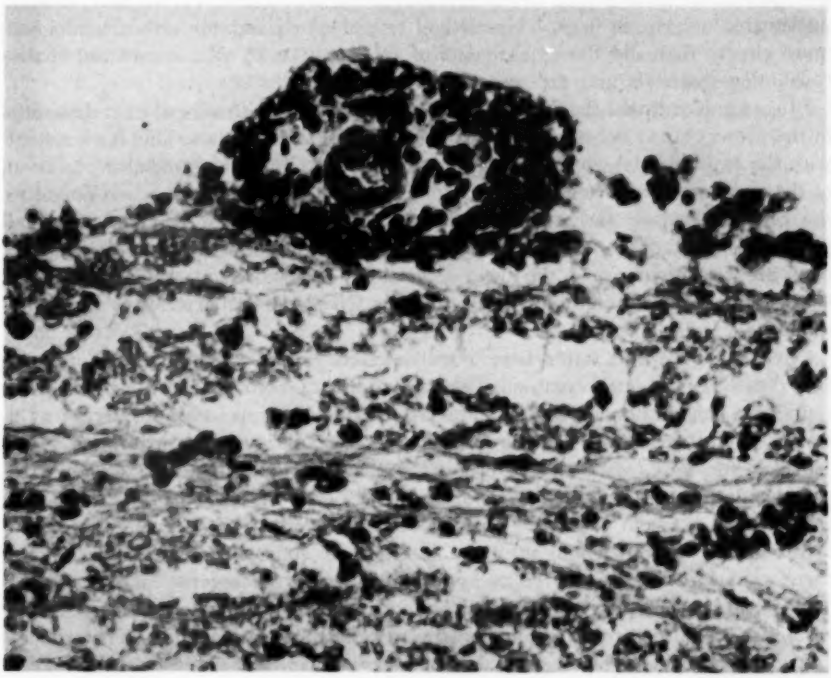


Fig. 5.—Photomicrograph showing the lining of one of the cavitations in Dog N-164-52. This animal had been killed 10 weeks after the injection of ethyl iodophenylundecylate into the cisterna magna. The margin is formed by compressed and partially necrotic spinal cord tissue without glial reaction. A blood vessel on the surface of the cavitation is surrounded by particles of India ink which had been injected into the fourth ventricle several hours before death. The presence of the India ink in the cavitation proves that direct communication existed from the ventricular system to the central canal and the cavitations. Hematoxylin and eosin stain; × 400.

COMMENT

Despite the acceptance of chronic adhesive arachnoiditis as a distinct clinical entity, relatively few reports of the associated spinal cord changes have appeared in the literature. As a result, considerable confusion has arisen in the past as to whether cavitation and adhesive arachnoiditis are part of the same process or whether one leads to the other. Charcot and Joffroy * were probably the first to

* Charcot and Joffroy, cited by Camus and Roussy.[8]

note the coexistence of these two lesions, and Joffroy and Achard † suggested that the cavitation was secondary to "hypertrophic cervical pachymeningitis." Subsequently, Philippe and Oberthur [2] expressed the opinion that the pachymeningitis was secondary to syringomyelia, the so-called pachymeningitic type of syringomyelia. A number of later writers,‡ however, have each reported one or more instances of myelomalacia associated with adhesive arachnoiditis and, in general, have agreed that cavitation results from vascular insufficiency induced by constriction of vessels in the course of the meningitic process.

Experimentally, Camus and Roussy [8] were able to produce pachymeningitis and spinal cord cavitation by the cisternal injection of a mixture of fatty acid, sodium nucleinate, and talc in dogs. This method resembled clinical adhesive arachnoiditis more closely than did the experiments of other workers § who introduced space-consuming materials into the spinal canal.

It is significant that the first evidence of degeneration with adhesive arachnoiditis in the present study occurred in the posterior horns and columns. This is consistent with the experimental work of Tauber and Langworthy,[11] who found that occlusion of the anterior spinal artery in cats by means of a ligature led to necrosis dorsal to the central canal. It is here that the terminal branches from both anterior and posterior spinal arteries are found and here that the first effects of vascular impairment are felt. Furthermore, the lower cervical region is more susceptible to ischemia because of the transition of supply in this area from vertebral arteries to segmental radicular vessels.

Syringomyelia-like states have been described in association with platybasia and Arnold-Chiari malformation. The cavitation has been attributed by Lichtenstein [12] to constriction of the vertebral arteries and venous stasis occurring as a result of deformity at the foramen magnum. In the experimental preparations under consideration here, some degree of hydrocephalus existed in almost all animals, and downward pressure may have contributed to the vascular insufficiency. That this was not the sole factor responsible for ischemia was shown by the extension of cavitation well down into the thoracic region in some animals and the lack of correlation between the degree of hydrocephalus and the severity of spinal cord damage. Moreover, the irregular distribution of the early spinal cord changes suggests that pressure at the foramen magnum was not the principal agent. The production of hydrocephalus in the animals of this series has been published elsewhere.[13]

It is beyond the scope of this report to enter the discussion of the pathogenesis of spontaneous syringomyelia. It has been suggested that this is a congenital defect resulting from ependymal cell rests which stimulate gliosis, which in turn leads to cavitation. However, it is noteworthy that there is frequently thickening of the meninges and of the vascular wall in the region of the syrinx. It seems possible, therefore, that the cavitation in spontaneous syringomyelia may sometimes have an ischemic basis. That it is not solely the result of ependymal hyperplasia is suggested by the lack of a complete ependymal lining to the syrinx. Indeed, the cavity of spontaneous syringomyelia with a partial ependymal lining may very closely resemble that which results from ischemia and which has become confluent with the central canal.

† Joffroy and Achard, cited by Camus and Roussy.[8]

‡ References 3, 4, 5, 6, and 7.

§ Lhermitte and Boveri.[9] Babes and D'Irsai, cited by Marinesco and Draginesco.[10]

The pathologic pattern of the material and the conditions leading to vascular insufficiency in our animals are similar to chronic adhesive arachnoiditis and its sequels as they occur in man. The spinal cord changes varied from small areas of gliosis and irregular cavitation to a syrinx comparable in size to that found in spontaneous syringomyelia. The myelomalacia produced in these animals confirms experimentally the ischemic origin of cavitation associated with chronic adhesive arachnoiditis. The term pseudosyringomyelia has been applied to this type of cavity but should not be used, since it might suggest a congenital basis for the lesion.

Many causes of chronic adhesive arachnoiditis are recognized. In the past, syphilis was the commonest but is of less importance today. Intrathecal administration of antibiotics, especially in excessive amounts, may lead to arachnoiditis. Other causes include occasional reactions to spinal anesthesia, repeated subarachnoid bleeding, and faulty technique during lumbar puncture. The etiology of many instances is still unknown. It is possible that, whatever the initiating factor may be, the resulting arachnoid thickening leads to ischemic changes in the spinal cord.

The similarity which exists between spontaneous syringomyelia and ischemic myelomalacia is of clinical significance. Congenital syringomyelia may occur without sensory changes,[12] whereas cavitation of vascular origin may present the classical sensory dissociation. This clinical similarity probably accounts for the variable results of treatment. It is evident also that, for patients with cavitation of vascular origin, roentgen ray therapy is not only unnecessary but may accentuate the underlying process.

In 13 animals in the series arachnoiditis developed as a result of ethyl iodophenylundecylate injection and 4 of these suffered damage to the spinal cord. This is insufficient evidence on which to condemn ethyl iodophenylundecylate as a clinical diagnostic agent, for the amount used in animals relative to the total cerebrospinal fluid volume was far in excess of that used in human patients. Furthermore, the variations in reactivity of human tissues and those of lower animals to foreign substances do not permit conclusions to be drawn on the basis of these experiments. Nevertheless, the observations again lend emphasis to the advisability of withdrawing as much ethyl iodophenylundecylate as possible after myelography.

SUMMARY

Chronic adhesive arachnoiditis was produced in dogs by the intracisternal injection of ethyl iodophenylundecylate (Pantopaque), kaolin, and the two agents in the same animal.

The arachnoiditis led to communicating hydrocephalus and also myelomalacia, with multiple cavitations in the cervicothoracic portion of the spinal cord. There was communication between the central canal and the cavitations.

The cavitations were judged to be due to ischemia resulting from compression of vessels in the meninges by the fibrosing arachnoiditis. The necrosis resulting from the ischemia was located at the junction of white and gray matter in the posterior portion of the spinal cord. It extended to include the posterior part of the ependyma of the central canal, thus allowing communication between the cavitation and the cerebrospinal fluid pathway.

The relation of the experimental findings to syringomyelia, chronic adhesive arachnoiditis, and vascular lesions of the spinal cord in clinical material was discussed.

NOTE.—Since this paper was completed, a publication [14] has appeared dealing with spinal cord cavitations associated with protrusions of intervertebral discs.

REFERENCES

1. Dixon, W. E., and Heller, H.: Arch. exper. Path. u. Pharmakol. **166**:265, 1932.
2. Philippe, C., and Oberthur, J.: Arch. méd. expér. et anat. path. **12**:513, 1900.
3. Davison, C., and Keschner, M.: Arch. Neurol. & Psychiat. **30**:1074, 1933.
4. Mackay, R. P.: J. A. M. A. **112**:802, 1939.
5. Lubin, A. J.: Arch. Neurol. & Psychiat. **44**:409, 1940.
6. Nelson, J.: Arch. Neurol. & Psychiat. **50**:1, 1943.
7. Vogel, P. J.: Bull. Los Angeles Neurol. Soc. **11**:58, 1946.
8. Camus, J., and Roussy, G.: Rev. neurol. **22**:213, 1914.
9. Lhermitte, J., and Boveri, P.: Compt. rend. Soc. biol. **72**:304, 1912.
10. Marinesco, G., and Draginesco, S.: Rev. neurol. **2**:338, 1924.
11. Tauber, E. S., and Langworthy, O. R.: J. Nerv. & Ment. Dis. **81**:245, 1935.
12. Lichtenstein, B. W.: Arch. Neurol. & Psychiat. **44**:792, 1940.
13. Schurr, P. H.; McLaurin, R. L., and Ingraham, F. D.: J. Neurosurg. **10**:515, 1953.
14. Mair, W. G. P., and Druchman, R.: Brain **76**:70, 1953.

VARYING CONTENT OF NUCLEIC ACIDS IN POLYMORPHONUCLEAR LEUCOCYTES IN ABSCESSES OF PATIENTS

M. L. MENTEN, M.D., Ph.D.
AND
MARY WILLMS, M.A.
VANCOUVER, B. C., CANADA

CHEMICAL analyses carried out by us on the nucleic acid in samples of bone marrow aspirated from patients with leukemia [1] and various blood dyscrasias* occasionally showed values of cellular deoxyribonucleic acid phosphorus (DNAP) below the range of 0.62 to 0.71 $\times$ 10^{-9} mg., figures which have been reported † as the approximate limits for this nuclear component in normal diploid cells of Mammalia. We have found that a considerable reduction in cellular DNAP might occur after x-irradiation of patients with leukemia.[1] The lower values commonly were not diffusely scattered but tended to fall into definite levels. It was thought that pus in abscesses where constituent neutrophiles were undergoing degradation due to the noxious action of toxins from phagocytosed bacteria might offer a medium for the study of reduction of DNAP in cells. Since the introduction into therapeusis of antibiotics and their early use in infections, considerable difficulty has been experienced in securing patients with abscesses containing sufficient purulent fluid for chemical analysis. Only 13 patients with fairly large abscesses have been available to us in the Vancouver General Hospital during the past year. Results of analysis of DNAP and RNAP of pus evacuated from abscesses in these 13 patients are presented in this paper.

Métais and Mandel,[7] in an analysis of DNAP of leukocytes in blood from 22 persons, 13 normal and 9 suffering from disease, reported a range of values lying between 6.45 and 7.37 $\times$ 10^{-7} γ with a mean of 6.92 $\times$ 10^{-7} γ of DNAP per cell. Little difference in value was observed, regardless of whether the leukocytes were derived from well or sick persons.

METHODS

The pus, removed from the incised abscess as aseptically as possible, was pipetted into a test tube and immediately sent to the laboratory. A small sample taken from about one-half of the specimens was also sent to the Department of Bacteriology in the Hospital for culturing of organisms and testing their sensitivity to antibiotics. Films of each specimen were stained with the Giemsa solution. Enumeration of the neutrophiles per cubic millimeter contained in each specimen was made with a Neubauer hemocytometer. Chemical analysis was performed according to the technique previously outlined.[2]

From the British Columbia Medical Research Institute and the Vancouver General Hospital.

This project was carried out under a grant from the British Columbia Division of the Canadian Cancer Society.

* References 2 and 3.

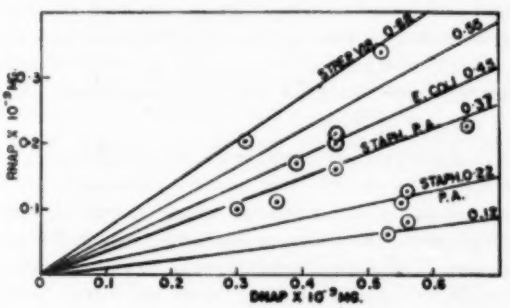
† References 4 to 6.

Patient	Age, Yr.	Site of Abscess	Bacteria Cultured	Sensitivity to Antibiotics*				DNAP, Mg. × 10^{-9} per Cell	RNAP, Mg. × 10^{-9} per Cell	Ratio, RNAP: DNAP	Comments
				Penicillin	Streptomycin	Chlortetracycline†	Chloramphenicol‡				
A. H.	..	Skin	Not cultured	..	..	..	..	0.53	0.06	0.12	Incision of abscess in OPD
A. B.	..	Skin	Not cultured	..	..	..	..	0.56	0.08	0.13	Incision of abscess in OPD
U. E.	..	Skin	M. aureus	R	..	..	..	0.55	0.11	0.21	Incision of abscess in OPD
D. D.	18	Rt. mandible	Hemolytic streptococcus, M. albus	S	S	S	S	0.56	0.13	0.23	Caries of right lower molar; alveolar abscess; pain of 1-week duration in lower right jaw
M. N.	34	Left thigh	M. aureus	S	R	S	S	0.36§	0.11	0.30	Penicillin and streptomycin given for 2 days before incision of abscess
R. B.	30	Left parotid	Not cultured	..	..	..	..	0.65	0.23	0.35	Abscess of left parotid gland; acute lymphadenitis of left posterior cervical lymph nodes
M. S.	84	Decubitus ulcer (thigh)	Str. mitis, Esch. coli ‖	S	S	R	R	0.90	0.10	0.36	Developed decubitus ulcer
J. H.	56	Abdomen, pelvis	M. aureus	S	S	S	S	0.45	0.16	0.36	Acute inflammation of diverticulum of sigmoid and pelvic abscess
J. S.	7	Appendix, pelvis	Esch. coli, Str. faecalis	..	..	S	S	0.45	0.20	0.44	Penicillin and chlortetracycline given for 2 days before operation; pathologic report on excised tissue; abscess of appendix; acute periappendicitis
C. S.	32	Appendix	Esch. coli	..	S	S	R	0.38	0.17	0.45	Small rectocele; early pyonephritis; dysuria; external hemorrhoids
P. K.	40	Ischiorectal tissue	Esch. coli	..	..	..	..	0.45	0.21	0.46	Pathologic report on tissue from incision; acute gangrenous appendicitis; acute inflammation of adherent omentum
B. C.	70	Urinary bladder	Str. mitis	S	S	S	S	0.51	0.20	0.65	Antibiotics given previous to suprapubic drainage
L. L.	6	Neck	Culture neg	..	..	..	..	0.52	0.34	0.67	Antibiotics given for 4 days before incision of abscess; retention of urine

* R, resistant; S, sensitive.
† Aureomycin.
‡ Chloromycetin.
§ Same specimen of pus after standing at room temperature three weeks gave identical values on analysis.
‖ Only a few Esch. coli, which were probably contaminant.

RESULTS

The amounts of DNAP and RNAP per cell, together with relevant data, are presented in the Table. The ratio of RNAP to DNAP gave a series of values which fell into five groups. The mean value of each group in ascending sequence was 0.12, 0.22, 0.34, 0.45, and 0.65, which formed the basis for the order of arrangement of data in the Table. Individual figures for ratios within each group vary somewhat, probably because of slight inaccuracies in cell counts on samples of pus which were very thick and tenacious. Values in each of the five groups were more or less correlated with the main bacterium responsible for the infection. Occasionally the bacteria obtained in cultures appeared to be persisting antibiotic-resistant organisms, rather than the primary pathogen, as in the case of Patient M. S., who had a decubitus ulcer. The bacteria in the samples of pus which were cultured were Micrococcus (Staphylococcus) pyogenes aureus, Escherichia coli, a hemolytic Streptococcus, Streptococcus faecalis, and Str. mitis. The two latter organisms may have



Relationship of RNAP to DNAP in neutrophiles of pus from abscesses in patients.

been gamma streptococci persisting after treatment of patients with antibiotics to which these bacteria were resistant. Variations in the reaction of the different strains of staphylococci were probably due to different degrees of resistance offered to various antibiotics, as seen by resistance to penicillin recorded for Patient U. E. Unfortunately, in our studies the number of strains of available staphylococci and their different reactions to antibiotics were limited in number.

The extent of degeneration in the pus cells of the various groups, demonstrable in Giemsa-stained films, roughly paralleled in reverse order the serial values of the RNAP to DNAP ratios. The lower the RNAP to DNAP ratio, the greater the degeneration of the pus cells. Staining was found to be almost exclusively intracellular.

The values per cell for each pair of related nucleic acids, DNAP and RNAP, have been plotted as points in a scatter diagram in which the abscissa x is DNAP $\times 10^{-9}$ mg., and the ordinate y is RNAP $\times 10^{-9}$ mg. per cell. These points fall along, or close to, five straight lines, which, when extended inward, end in zero. Each of the straight lines corresponds to a group characterized by a definite ratio of RNAP to DNAP, as detailed in serial figures in the Table.

COMMENT

Although only a few specimens of pus from patients were available for study, the results as analyzed in the scatter diagram of the accompanying graph indicate, we believe, trends which throw light on the relationship of RNA to DNA in the cell. As seen in the Chart, the position of the points representing pairs of the plotted variables, DNAP and RNAP, decreased in each line of regression, depending upon the extent to which the deterioration of the nucleic acids had progressed. The deterioration in our material depended on such unfavorable environmental conditions as the number of bacteria, their toxins, and the time over which they had acted. Other factors, such as acidity and electrolytic imbalance developing during the formation of pus, may also contribute to the degradation of the pus cell. In a chemical analysis of empyemal pus previously undertaken but not completed by one of us (M. L. M.), it was found that the reaction of pus evacuated from the walled-off empyemal sacs might be increased to pH 5 or 4, with a concomitant rise in the phosphate and ammonium salt content and a reduction in sodium and potassium. The complex nature of DNA in pus, its continuously changing composition by enzymic degradation both before and after therapy, has been extensively investigated by Tillett and his associates. A brief review of Tillett's work, with references, has been recorded by Sherry and Goeller [8] in a paper in which a tentative scheme of possible nucleolytic enzymes and their successive breakdown products was suggested. The RNA was not included.

The relationship of the two correlated variables, DNAP and RNAP, is represented by the equation for a straight line, namely $y=a+bx$. In the Chart the a, or the y intercept, is 0, and x and y are the values obtained by chemical analysis; so that b, the coefficient of correlation, is a constant which indicates the slope of each line of regression. The slope of each line, as already noted, is therefore the ratio of RNAP to DNAP. Mean values obtained for b tend to be slightly lower than those we have found in other similar studies. It is also probable from analogous data obtained by us that, instead of an average figure of 0.34 for b in the line of regression occurring with penicillin-sensitive Staphylococcus, there should be two values, namely, 0.30 and 0.37. Increase in value of b in each successively higher line of regression is due in large part to a relatively greater increase in the figure obtained for cellular RNAP, as compared to that of cellular DNAP. The accuracy of b in individual analyses was between 5 and 10%. Inaccuracies were due mainly to difficulty in obtaining an exact figure for cell counts per cubic millimeter in different samples of pus. The extent to which the reduction of the two nucleic acids had progressed was limited to a considerable degree by antibiotic treatment, which the patient invariably received previous to incision of the abscess. Bacteria in which noxious effects are unrestrained by antibiotic therapy can probably produce a maximum decrease in the nucleic acid content of the pus cells, although other factors operative in pus, which were previously mentioned, may exert modifying influences. The type of bacterium and its elaborated toxins seem to exhibit a more or less specific effect in determining the degree of dissolution of nuclear contents.

Although the analyses on the pus cell are relatively few in number, the results are in accord with our other studies of a similar nature. As is shown in other studies, the correlation of RNAP and DNAP outlined for pus cells are not unique for neutrophiles but are found in other mammalian cells.

SUMMARY

The deoxyribonucleic acid phosporus and ribonucleic acid phosphorus content per cell of neutrophiles undergoing devolution in abscesses showed definite relationships, which could be demonstrated graphically in a scatter diagram in which DNAP was abscissa and RNAP was ordinate. The lines of regression which were developed were related to the bacteria which gave rise to the infection.

The slope of the various lines of regression was determined by the equation for a straight line, $y=a+bx$. Serial values of b, the coefficient of correlation, representing the ratio of RNAP to DNAP in the five lines of regression, were 0.12, 0.22, 0.34, 0.45, and 0.65.

REFERENCES

1. Menten, M. L., and Willms, M.: Nucleic Acid Content of Cells of Bone Marrow Aspirated from Patients with Leukemia, Cancer Res. **13**:733-736, 1953.

2. Menten, M. L., and Willms, M.: Changing Values of Nucleic Acid of Cells of Bone Marrow During Development of Red Blood Cell, A. M. A. Arch. Path. **54**:343-350, 1952.

3. Menten, M. L., and Willms, M.: Nucleic Acid of Cells of Bone Marrow of Patients with Pernicious Anemia, A. M. A. Arch. Path. **54**:351-358, 1952.

4. Mirsky, A. E., and Ris, H.: Variable and Constant Components of Chromosomes, Nature, London **163**:666-667, 1949.

5. Kurnick, N. B.: Quantitative Estimations of Desoxyribose Nucleic Acid Based on Methyl Green Staining, Exper. Cell Res. **1**:151-158, 1950.

6. Vendrely, R., and Vendrely, C.: La teneur du noyau cellulaire en acide désoxyribonucleique à travers les organes, les individus, et les espèces animales; Etude particulière des mammifères, Experientia **5**:327-329, 1949.

7. Métais, P., and Mandel, P.: Teneur en acide désoxypentosenucléique des leucocytes chez l'homme normal et à l'état pathologique, Compt. rend. Soc. biol. **144**:277-279, 1950.

8. Sherry, S., and Goeller, J. P.: The Extent of Enzymatic Degradation of Desoxyribonucleic Acid (DNA) in Purulent Exudates by Streptodornase, J. Clin. Invest. **29**:1588-1594, 1950.

IN VITRO DEHYDROGENASE ACTIVITY OF ADRENAL SLICES IN CONTROL AND TUMOR-BEARING MICE

MAURICE M. BLACK, M.D.
AND
FRANCIS D. SPEER, M.D.
NEW YORK

PREVIOUS reports from this laboratory have described the use of tetrazolium salts to measure the intensity of the dehydrogenase activity of surviving tissue slices.* In addition, it was shown that the site and relative intensity of such activity could be visualized by utilizing the tetrazolium salts as a histochemical tool.† This latter procedure appeared to be of decided advantage in providing evidence of metabolic activity in the adrenal cortex of the rat, dog, and man in health and disease.‡

In view of various reports in the literature in regard to alterations in adrenal function in tumor-bearing animals and patients, it appeared of value to study the dehydrogenase activity of the adrenals in control and tumor-bearing animals. Further, it was considered worth while to evaluate what differences, if any, could be noted in the metabolic activity of adrenals from male and female animals.

The data obtained indicate that there is a decided difference between the histochemically demonstrated dehydrogenase activity in the adrenals of male and female CFW, C_3H, and C_{57} black control mice. Further, the presence of a spontaneous breast tumor in female mice of the CFW and C_3H strains was associated with well-defined changes in the adrenal dehydrogenase activity as compared with the female controls. Similar changes were not observed in mice bearing implanted sarcoma 180. The alterations that did take place in the latter group were of the type observed in response to severe stress.

MATERIALS AND METHODS

In the Table we have indicated the numbers and types of mice employed in the present investigation. The mice were killed by crushing the cervical cord, weighed, and the adrenals were removed. The adrenals were sliced in half, and one half was placed in a wide-mouthed test tube containing 3 ml. of 1% aqueous solution of 2,3,5-triphenyltetrazolium chloride (TTC) and 1 ml. of normal saline buffered to pH 7.2. The tubes were incubated at 37 C. for one hour. Continuous gentle agitation was maintained throughout the incubation period. The reaction

From the Department of Pathology, New York Medical College, Flower and Fifth Avenue Hospitals.

This investigation was supported by the Damon Runyan Memorial Fund and the Helen Andreadis Foundation, Inc.

* References 1 to 4.

† References 4 to 6.

‡ References 7 and 8.

was stopped by decanting the incubation medium and fixing the tissues in 10% formalin. The adrenals were then sectioned on the freezing microtome, and the sections were examined for the relative intensity of TTC reduction in the different zones. In some instances counterstaining with polychrome methylene blue was carried out to provide better definition of the cellular detail. The remaining half of the adrenal gland was fixed in 10% formalin. From this half, frozen sections were prepared and stained for lipid, using Sudan IV. In addition, routine sections stained with hematoxylin and eosin were made from the remaining tissue and the other adrenal gland.

RESULTS

In view of the lack of any appreciable difference in the appearance of the adrenals from CFW, C_3H, and C_{57} black mice under the various conditions to be described, the data obtained will be presented collectively unless otherwise indicated.

Hematoxylin and Eosin Stained Adrenals.—Microscopic sections of adrenals of male and female control mice prepared in a routine manner and stained with hematoxylin and eosin showed only a poor differentiation between the glomerulosa and fasciculata or the fasciculata and reticularis. Aside from a slightly greater vacuo-

Groups of Mice Employed in Study of Adrenal Dehydrogenase Activity

Strain	Group *	Sex	No.	Body Wt., Gm., Mean	Tumor Wt., Gm., Mean
CFW.....	Control	F	6	25.7	—
	Control	M	6	23.6	—
	T_B	F	11	35.3	1.2
	T_{B180}	M	6	17.6	1.9
	T_{B180}	F	4	20.8	2.7
C_3H.....	Control	F	4	22.7	—
	Control	M	5	21.4	—
	T_B	F	6	31.2	1.9
C_{57} black.....	Control	F	4	21.6	—
	Control	M	4	22.8	—

* T_B, spontaneous mammary tumor; T_{B180}, implanted sarcoma 180.

lation of the cells of the outermost cortex in the female controls, there was no appreciable difference discernible between the adrenals from the male and the female control mice (Figs. 1 and 2). A similar appearance was also observed in the adrenals from female mice bearing spontaneous mammary tumors (Fig. 3). In the case of mice bearing implanted sarcoma 180, it was noted that there tended to be a reduced vacuolation of the cells of the inner zones of the adrenal cortex, but no appreciable distinction could be made between the cells of the glomerulosa and outer fasciculata (Fig. 4). In short, the appearance of the adrenal sections prepared in the routine fashion gave no indication of any real difference in functional activity between the different areas of the same glands or any reflection of the sex of the animal or the presence or absence of a spontaneous or implanted tumor.

Sudan-Stained Frozen Sections.—Lipid-stained sections of the adrenals revealed no appreciable difference in the appearance of the glomerulosa of the adrenals from the male and the female controls or the adrenals from females bearing spontaneous tumors. There was some tendency for the lipid staining to be somewhat less prominent in the fasciculata zone of the adrenals from the male mice, but this was neither striking nor constant. However, the lipid staining in the glomerulosa and reticularis zones in these three groups was definitely less than in the fasciculata (Figs. 5 and 6). In contrast to the well-defined sudanophilia observed in the above-described

adrenals, there was a loss of sudanophilia in the adrenals from male and female mice bearing sarcoma 180. This loss of sudanophilia was similar to that described by Dalton [9] and was most marked in the inner zones of the cortex. The staining was limited in extreme cases to the glomerulosa and a few layers of cells of the outermost fasciculata.

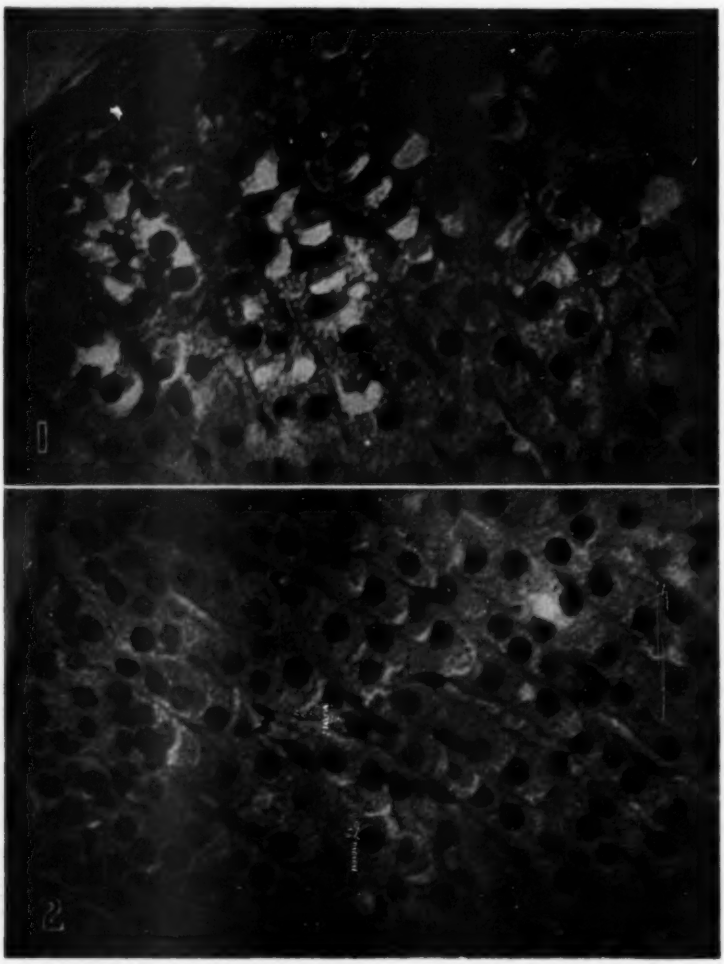


Fig. 1.—Adrenal gland of CFW female control mouse; glomerulosa and outer fasciculata; hematoxylin and eosin.

Fig. 2.—Adrenal gland of CFW male control mouse; glomerulosa and outer fasciculata; hematoxylin and eosin.

TTC Reduction.—Female Controls: The histochemical appearance of adrenal slices from control female mice after incubation in TTC was characterized by a prominent red staining of the intracytoplasmic lipid droplets in the cells of the

fasciculata. In contrast, the glomerulosa was unstained and was thus sharply delimited from the fasciculata. The innermost zone of the cortex was also unstained, although lipid-containing cells were readily demonstrable in this area (Figs. 7 and 8).

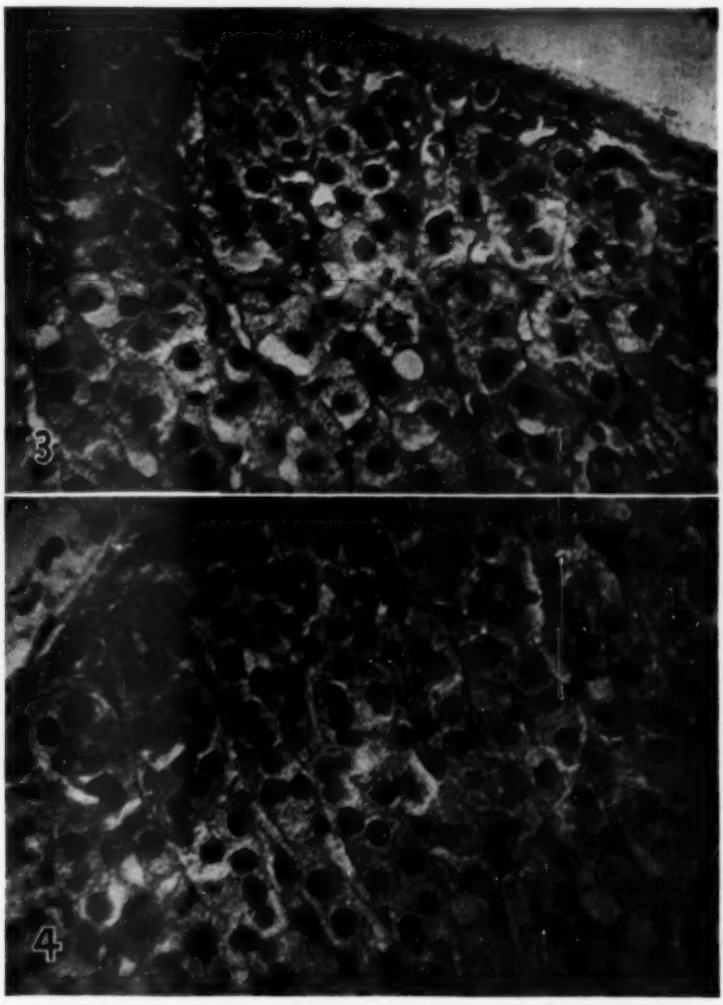


Fig. 3.—Adrenal gland of CFW female mouse with a spontaneous mammary carcinoma; glomerulosa and outer fasciculata; hematoxylin and eosin.

Fig. 4.—Adrenal gland of CFW female mouse with an implanted sarcoma 180; glomerulosa and outer fasciculata; hematoxylin and eosin.

Male Controls: The formazin deposition in the adrenal slices from male mice occurred in the lipid-laden cells of the cortex. The appearance was thus similar to

that obtained with the Sudan stain, although the glomerulosa staining was more prominent than with the Sudan stain. In contrast to the female control, the glomerulosa and reticularis region showed active reduction of the TTC. In addition, variable amounts of rod-like formazin crystals were noted in the cytoplasm of the cells of the reticularis. It should also be noted that the intensity of the red staining of the fasciculata was somewhat less in the slices from the males than it was in the case of the female mice.

Females with Spontaneous Mammary Tumors: The reduction of TTC by adrenal slices from the female mice with spontaneous tumors was somewhat more variable than that of the female or male controls. The appearance in the majority of instances was similar to that of the male controls, although the glomerulosa staining

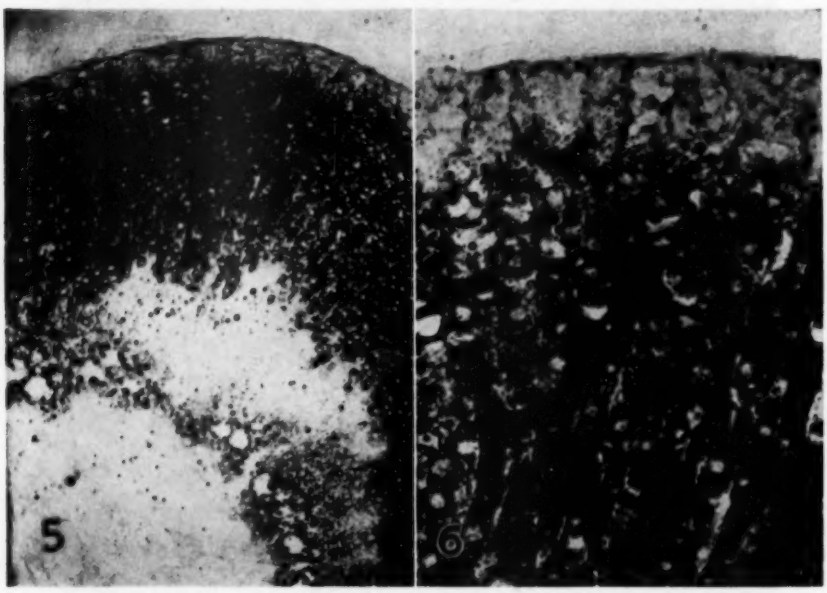


Fig. 5.—Adrenal gland of CFW female control mouse. This appearance is essentially similar to that of the male CFW control and the CFW female with a spontaneous mammary tumor; Sudan IV.

Fig. 6.—Adrenal gland of CFW female control mouse; glomerulosa and outer fasciculata;

was not always as prominent as in the latter group. It should also be mentioned that the staining in the fasciculata region, while similar to that of the female controls, appeared somewhat more intense. In general, it might be said that reduction by adrenal slices from the spontaneous tumor-bearing female mice tended to be so similar to that of the male controls in regard to the site and intensity of staining as to be almost indistinguishable in the majority of cases (Figs. 9 and 10).

Male and Female CFW Mice with Implanted Sarcoma 180: The histochemical appearance of the adrenals of male and female mice bearing sarcoma 180 showed variable degrees of change from the normal controls' appearance, ranging from no apparent change to a picture identical with that of a severe stress reaction. The first

change noted in the present series was a loss of TTC reduction by the inner zones of the cortex. This loss of intracellular TTC reduction progressed peripherad, so that eventually the intracellular staining was confined to a small portion of the outer fasciculata in the female and the outer fasciculata and glomerulosa in the male. Such

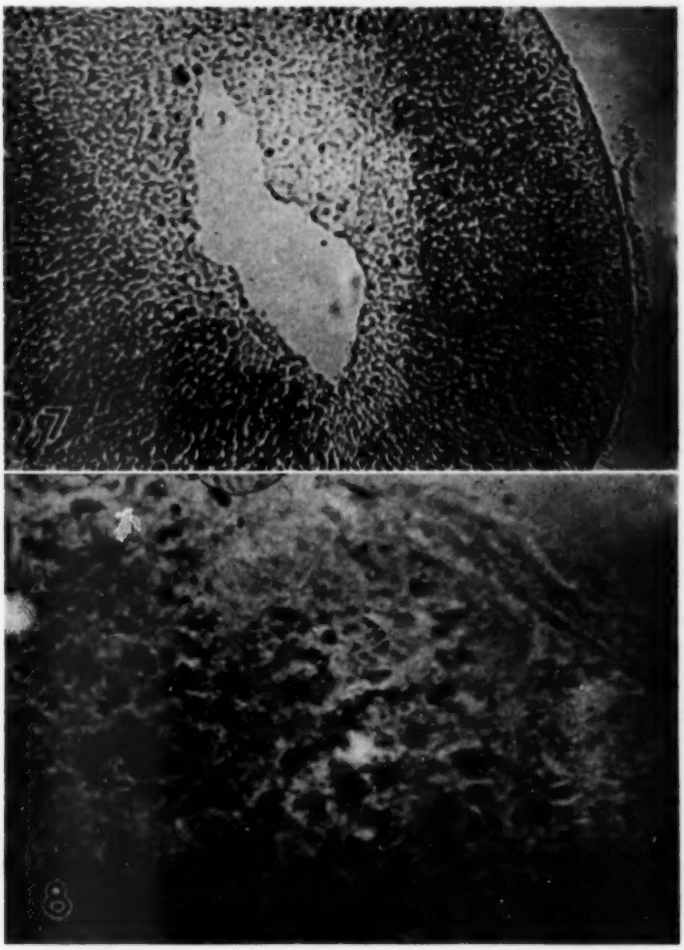


Fig. 7.—Adrenal gland of C$_3$H female control mouse after incubation in TTC. Note limitation of the staining to the fasciculata zone.

Fig. 8.—Adrenal gland of CFW female control mouse; glomerulosa and outer fasciculata; TTC incubation. Note lack of reaction in glomerulosa zone.

changes were most evident in moribund mice with large tumors. It should be mentioned that in some instances the glomerulosa zone of such depleted adrenals from females with sarcoma 180 reduced the TTC and thus appeared similar to the adrenals of the males with the implanted tumor. In addition, scattered extracellular deposits

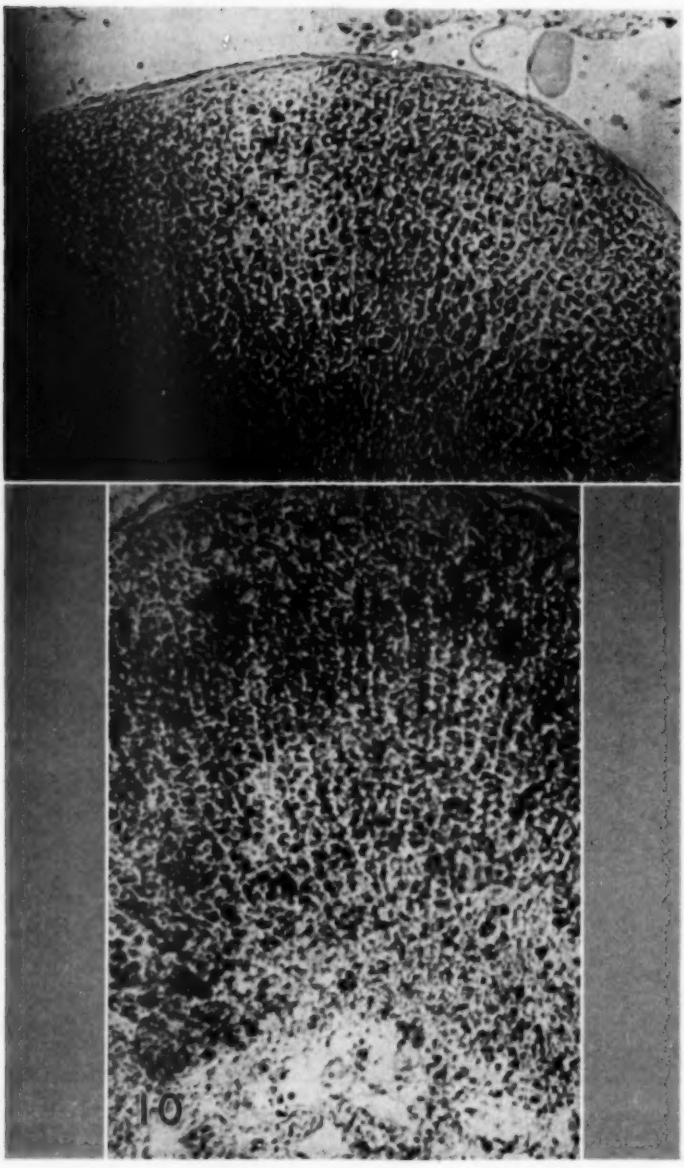


Fig. 9.—Adrenal gland of C_3H female with spontaneous mammary carcinoma; TTC incubation. Note staining throughout cortex. The appearance of male C_3H control adrenal is essentially similar to the above.

Fig. 10.—Adrenal gland of CFW female with spontaneous mammary carcinoma; TTC incubation. Note staining throughout cortex. The staining of the male CFW control is essentially similar to the above.

of formazin crystals were aiso noted throughout the cortical zones (Figs. 11 and 12). Associated with the loss of TTC reduction, there was a loss of intracellular lipid which also appeared to start in the inner zones and progress peripherad. However, it should be mentioned that the change in dehydrogenase activity preceded the loss

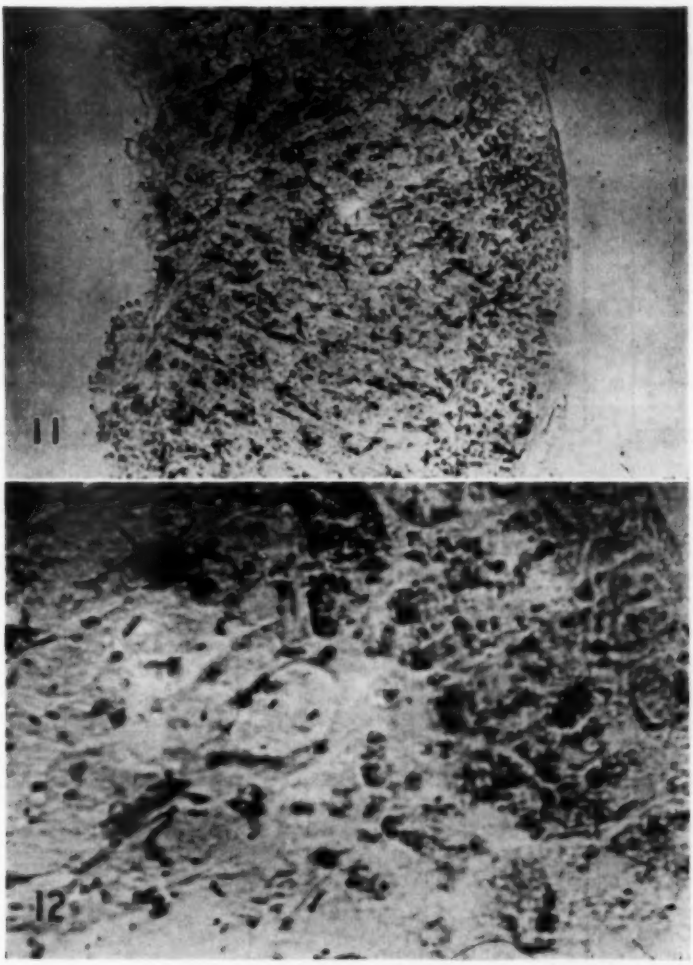


Fig. 11.—Adrenal gland of CFW male with implanted sarcoma 180; TTC incubation. Note the limitation of intracellular TTC reduction to outer cortex and the presence of extracellular formazin crystals.

Fig. 12.—Adrenal gland of CFW female with implanted sarcoma 180; TTC incubation; glomerulosa and outer fasciculata. Note that the intracellular staining is almost exclusively in the outermost fasciculata region.

of intracellular lipid droplets, as evidenced by finding minimal or no evidence of TTC reduction in cells still containing appreciable lipid.

COMMENT

It is evident from the foregoing, that routine hematoxylin and eosin stained sections do not adequately reflect adrenal cytophysiology in either normal or abnormal states. It is this inadequacy which has led to the use of lipid staining and other specialized techniques to more adequately analyze the functional activity of the adrenal cortex.§ However, as indicated in the present study, it has proved difficult to measure objectively the dynamic processes of cell secretory activity solely on the basis of criteria such as the intensity and character of the lipid distribution. What appears to be lacking is some additional parameter which would provide a direct

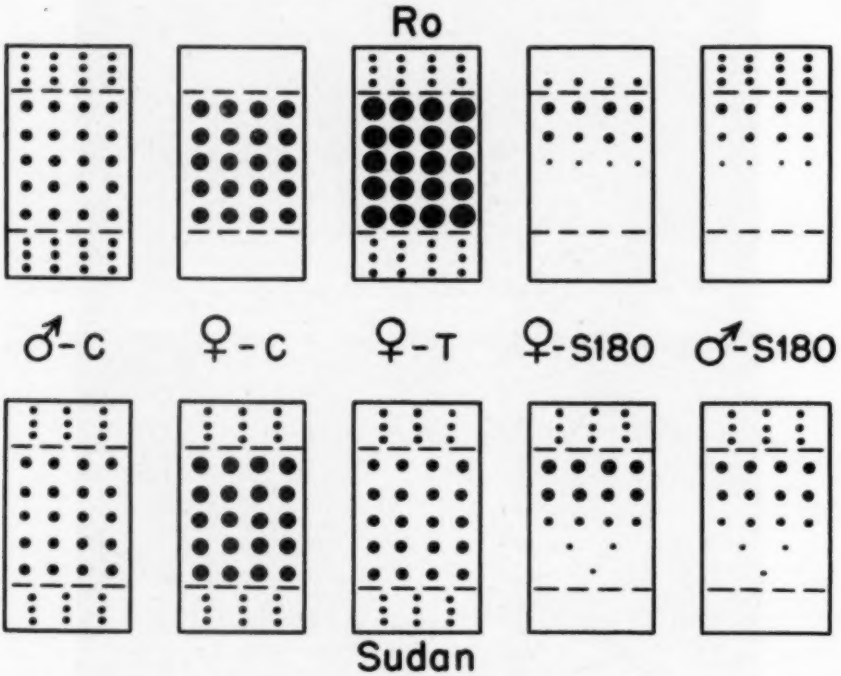


Fig. 13.—Diagrammatic representation of dehydrogenase activity and lipid content of adrenal cortex in control and tumor-bearing mice.

measure of the metabolic activity of the adrenal cortex. The data obtained in the present investigation, as well as our previous studies, would indicate that the histochemical use of TTC might provide just such information. In Figure 13 we have diagrammed the pertinent findings observed in regard to sudanophilia and dehydrogenase activity of the adrenal slices studied. These data indicate that the TTC reduction by adrenal slices from male control mice paralleled the lipid content (sudanophilia) of the cortical cells. Similarly in the adrenals from mice bearing implanted sarcoma 180 there was a coincident loss of sudanophilia and intracellular TTC

§ References 10 to 13.

reduction. It should be emphasized, however, that the loss of TTC reduction by the cortical cells preceded the lipid depletion. On the other hand, a dissociation between sudanophilia and TTC reduction was found in the adrenals of the female control mice. Here the dehydrogenase activity of the glomerulosa and reticularis was minimal, although sudanophilia was present in amounts similar to those observed in the male controls. These findings indicate that the histochemical demonstration of the dehydrogenase activity of the adrenocortical cells is not necessarily related to the lipid content of the cell and appears to be a more critical index of function than sudanophilia.

The present data does not allow of interpretation as to the exact significance of the differences in the dehydrogenase activity of the different cortical zones as observed in adrenal slices from the male and female animals. It is possible, however, that the tetrazolium technique might be applicable to a more adequate evaluation of the differential sex incidence and behavior of certain systemic diseases which appear to have an adrenal component, viz., lupus erythematosus, hypertension, etc.

Of equal interest is the observation that, in regard to zonal metabolism, the dehydrogenase activity of the adrenals of female mice bearing spontaneous mammary tumors differed markedly from the control females and tended to be similar to that found in the males. Such changes do not appear to be the result of a nonspecific stress situation or due to the mere physical presence of a growing tumor mass, since no such effect was observed in mice implanted with sarcoma 180. It should also be noted that Dalton [9] failed to observe any consistent change in the lipid content of the adrenals of mice bearing spontaneous tumors. Nor did our observations reveal any lipid depletion in the mice bearing spontaneous mammary tumors. The change in the adrenals of spontaneous-tumor mice is thus a complex one. On the one hand there was a marked increase in the dehydrogenase activity of the glomerulosa and reticularis and a smaller increase in the dehydrogenase activity of the fasciculata as compared with the female controls. On the other hand, there was no appreciable change in the lipid content of the adrenocortical cells. Such findings are consistent with the concept that the presence of a spontaneously arising malignant tumor is associated with alterations in adrenal function which are more properly classified as dysfunctions rather than adrenal exhaustion.‖

In contrast, the findings in adrenals of the transplantable tumor group were consistent with adrenal exhaustion. The appearance of these glands in regard to TTC reduction was almost identical to that previously described by us in the adrenals of rats subjected to cold stress or acute corticotropin (ACTH) discharge and in extreme cases to the reaction of the gland in hypophysectomized animals. The differences in the metabolic activity of the adrenal cortex in the presence of spontaneous as compared with transplanted tumors provide further indication of the fundamental dissimilarity of the two situations and indicate the need for caution in applying the data from one to the other.

Finally, it is significant to note that the various data presented indicate that there are clear-cut differences in metabolic activity and presumably function of the various zones of the adrenal cortex. These findings confirm our previous observations on the adrenals of rats, dogs, and humans and provide further indication of the value of the tetrazolium salts as a histochemical tool in investigating adrenal functional changes.

‖ References 14 and 15.

SUMMARY

A tetrazolium salt (2,3,5-triphenyltetrazolium chloride) was used as a histochemical tool to visualize the in vitro dehydrogenase activity of the adrenals from CFW, C_3H and C_{57} black mice. A comparison was made of the dehydrogenase activity of the adrenals from control male and female mice and mice bearing spontaneous mammary tumors and implanted sarcoma 180. The present study revealed that the adrenals of the male mice were characterized by a greater dehydrogenase activity of the glomerulosa and reticularis zones than the female controls. The presence of a spontaneous mammary tumor in the female mice was associated with an adrenal dehydrogenase activity similar to that of the male. In contrast, implanted sarcoma 180 was associated with a decrease in the dehydrogenase activity of the reticularis and inner fasciculata zones.

The TTC preparations were compared with Sudan stains and routine hematoxylin and eosin preparations in regard to their ability to reflect the functional activity of the adrenal gland. Our findings would indicate that of the three methods of study, the TTC technique is the most sensitive index of adrenal function and should prove to be a valuable adjunct in the study of adrenal alterations under diverse conditions.

REFERENCES

1. Black, M. M., and Kleiner, I. S.: Science **110**:660, 1949.

2. Black, M. M., and Kleiner, I. S.: Proc. Soc. Exer. Biol. & Med. **76**:437, 1951.

3. Black, M. M., and Speer, F. D.: Am. J. Clin. Path. **23**:218, 1953.

4. Black, M. M.; Zweifach, B. W., and Speer, F. D.: Am. J. Clin. Path. **23**:332, 1953.

5. Black, M. M.; Kleiner, I. S., and Speer, F. D.: Proc. Soc. Exper. Biol. & Med. **77**:206, 1951.

6. Zweifach, B. W.; Black, M. M., and Shorr, E.: Proc. Soc. Exper. Biol. & Med. **74**:848, 1950.

7. Zweifach, B. W.; Black, M. M., and Shorr, E.: Proc. Soc. Exper. Biol. & Med. **76**:446, 1951.

8. Shorr, E.; Zweifach, B. W., and Black, M. M.: Tr. A. Am. Physicians **64**:445, 1951.

9. Dalton, A. J.: J. Nat. Cancer Inst. **5**:99, 1944.

10. Dempsey, E. W.: Recent Progr. Hormone Res. **3**:127, 1948.

11. Greep, R. O., and Deane, H. W.: Ann. New York Acad. Sc. **50**:596, 1949.

12. Sayers, G.: Physiol. Rev. **30**:241, 1950.

13. Selye, H., and Stone, H.: On the Experimental Morphology of the Adrenal Cortex, Publication No. 74, American Lecture Series, Monograph in American Lectures in Endocrinology, Springfield, Ill., Charles C Thomas, Publisher, 1950.

14. Lieberman, S., and Dobriner, K.: Recent Progr. Hormone Res. **3**:71, 1948.

15. Reifenstein, E. C., Jr.; Duffy, B. J., Jr., and Grossman, M. S.: Gastroenterology **13**:493, 1949.

Case Reports

EMBRYOLOGICAL ANEURYSM OF THE MYOCARDIAL VESSELS

WILLIAM V. LOVITT Jr., M.D.
BALTIMORE
AND
MAJOR STANLEY LUTZ Jr.
MEDICAL CORPS, UNITED STATES AIR FORCE

CONGENITAL aneurysms of the ventricular myocardium are an extremely rare occurrence. Consequently, only a few malformations of the vascular supply of the heart are reported in the literature. No previous abnormality of this type in an adult has been reported.

REPORT OF CASE

W. A., a 62-year-old white man, was found dead by his wife in the garden on the evening of Aug. 26, 1952. There was no evidence of violence, and when last seen by his wife the deceased appeared to be well and had no complaints. He had been employed for a number of years as a paint sprayer and was retired in 1945. The decedent's symptoms over a period of several years prior to his sudden death indicated that he had been suffering from long-standing hypertension, although there was no history of hospitalization or medical attention subsequent to his retirement. At autopsy the chief pathologic alterations were confined to the heart, which was enlarged, weighing 500 gm. There was extensive hypertrophy of the left ventricle, which measured 2 cm. in thickness, and on the anterior surface of the left ventricle there was a rounded fibrous protrusion 2 cm. in diameter, which on section revealed a honeycomb cavity filled with blood. The cavity of the aneurysm communicated by an endothelial-lined channel directly with the lumen of the left ventricle. Multiple sections of the myocardium revealed no gross evidence of fibrosis. The coronary arteries, although mildly sclerotic, showed no areas of occlusion. The remainder of the heart showed no gross abnormalities. The lungs, liver, kidneys, and gastrointestinal tract showed no gross abnormalities.

Microscopic Examination.—The myocardium showed hypertrophy of the muscle fibers, and, extending from the lumen of the ventricle into the honeycomb aneurysm on the epicardial surface, there was a thin-walled endothelial-lined channel. The aneurysm was composed of numerous endothelial-lined cavities showing sclerotic change and was surrounded by extensive fibrosis. Within the walls of these cavities there was extensive elastic tissue. Entering the aneurysm were several branches of the left anterior descending coronary artery. Surrounding the aneurysm there was a thick well-organized fibrosis wall which extended into and around the superficial muscle fibers on the epicardial surface. Microscopic examination of the remainder of the organs showed no abnormalities, and death was ascribed to hypertensive cardiovascular disease.

COMMENT

The epicardial aneurysm and its endothelial-lined communication with the left ventricular cavity appeared to be a developmental defect in the wall of the ventricle.

Dr. Lovitt is Assistant Professor, Division Legal Medicine, University of Maryland School of Medicine and College of Physicians and Surgeons.

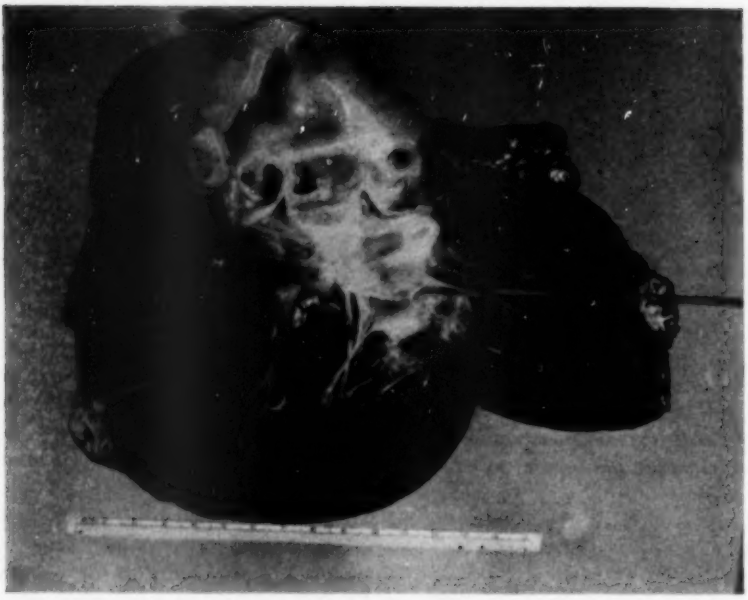


Fig. 1.—The honeycomb aneurysm is near the tip of the left ventricle. Note the absence of other myocardial alterations.

Fig. 2.—Note the large endothelial-lined channel communicating with the lumen of the ventricle.

This is supported by the absence of fibrosis, inflammation, or other alteration of the ventricular musculature. Also, the sclerotic change within the aneurysmal cavity indicated a long-standing defect.

The etiology of this defect can be explained by an abnormal embryological development of the vascular supply of the myocardium. A study of the embryological formation of the myocardial vessels by Wilson and Grant * showed them to be derived from two sources. One source is protusion of endothelial-lined channels from the ventricular cavity into the intertrabecular spaces. The other

Fig. 3.—Low-power enlargement of a microscopic section of the aneurysm. Elastic hematoxylin and eosin stain; × 16.

vessels arise by branching of the developing coronary arteries and veins. These two vascular systems communicate to form the vascular network of the heart. The outermost intertrabecular spaces, while at first relatively large and extending to the epicardial surface, become narrowed by continued development of the myocardium into capillary tubes which later are joined by the coronary vessels extending inward from the epicardium. Thus, there is formed in the cortical myocardium a vascular network composed of elements from both the coronary vessels and the intermuscular spaces. These intertrabecular vessels retain their ventricular com-

* References 1 and 2.

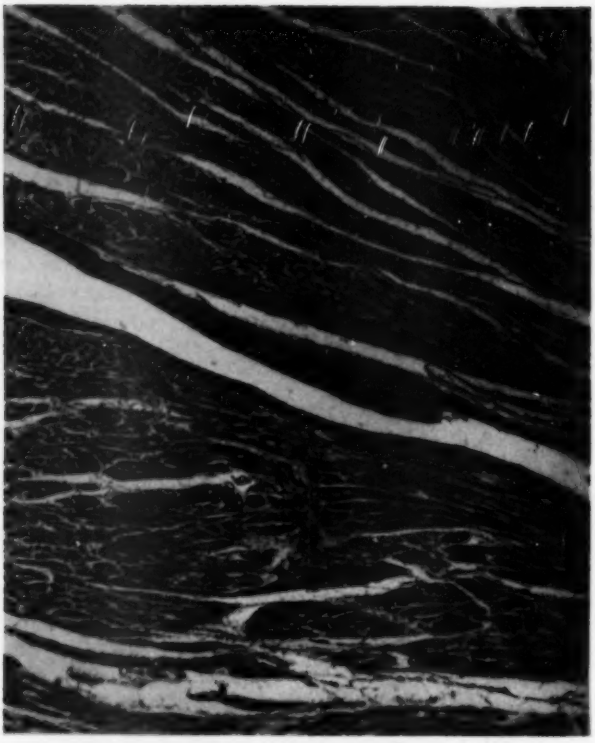


Fig. 4.—Section showing the endothelial-lined channel passing through the myocardium into the cavity of the left ventricle. Hematoxylin and eosin; × 140.

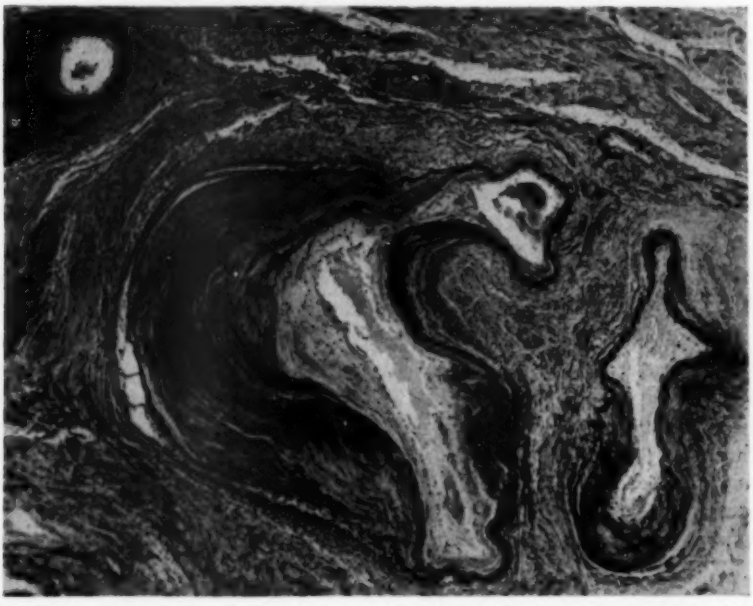


Fig. 5.—Low-power photomicrograph showing the extensive fibrosis surrounding the channels of the aneurysm. Also note the sclerotic change in the wall of these vascular spaces and the abundance of elastic tissue. Elastic stain; × 140.

munications to produce the Thebesian vessels of the adult heart. The importance of the Thebesian vessels was stressed by Wearn [3] and Spalteholz,[4] who showed that a large percentage of the coronary flow passes through them into the cavity of the ventricle.

Other congenital defects of the vasculature have been described. Mann, as quoted by Grant,[5] reported a case of a malformed heart in which there were wide intermuscular spaces. Schauder [6] described an ox heart in which there was a sac-like aneurysm of the left coronary artery with branches into the surrounding muscle but no communication with the ventricular cavity. Grant [5] reported a case of a malformed heart in a 14-month-old child, in which, among other congenital abnormalities, there was dilatation of the Thebesian vessels within the ventricular myocardium communicating freely with the ventricular cavity, as well as the coronary vessels. Accompanying these malformations, there was a sacular epicardial aneurysmal cavity communicating by numerous endothelial-lined intermuscular spaces with the cavity of the ventricle.

Theoretically, therefore, the interpretation of the present anomaly becomes possible; the opening from the ventricle communicating with the epicardial aneurysm represents an enlarged Thebesian vessel or embryonic sinusoid normally developed there. The persistence of the epicardial spaces forming the bulk of the aneurysm can be explained as a growth of the intertrabecular spaces which normally become reduced to capillaries and anastomose with the coronary vessels.

SUMMARY

This abnormality had no direct relation to the cause of death but presents an interesting embryologic malformation and serves to emphasize the relationships of the various vascular structures of the myocardium. By magnification of these developmental vascular structures, this malformation recapitulates the embryologic formation and connections of the Thebesian veins with the coronary vessels.

700 Fleet St. (Dr. Lovitt).

REFERENCES

1. Wilson, J. G., and Grant, R. T.: Congenital Malformation of the Heart in an Infant Associated with Partial Heart Block, Heart **12**:295, 1926.

2. Grant, R. T.: Development of the Cardiac Coronary Vessels in the Rabbit, Heart **13**:261, 1926.

3. Wearn, J. T.: The Rôle of the Thebesian Vessels in the Circulation of the Heart, J. Exper. Med. **47**:293, 1928.

4. Spalteholz, W.: Die Thebesischen Venen, Anat. Anz. **79**:212, 1934.

5. Grant, R. T.: An Unusual Anomaly of the Coronary Vessels in the Malformed Heart of a Child, Heart **13**:273, 1926.

6. Schauder, W.: Bisher unbekannte Missbildung der Arteria coronaria sinistra des Rindes, Anat. Anz. **58**:540, 1924.

Laboratory Methods and Technical Notes

METHOD FOR ROUTINE HISTOPATHOLOGIC STUDY OF HUMAN SINOATRIAL NODE

MAURICE LEV, M.D.
MIAMI BEACH, FLA.
AND
ALVIN L. WATNE, M.D.
CHICAGO

MATERIALS AND METHODS

The region of the superior part of the sulcus terminalis and the superior wall of both atria in 67 formalin-fixed normal hearts was dissected grossly. This region was then studied in 18 similar hearts histologically in both longitudinal and transverse planes. These studies yielded a preliminary concept of the location of the sinoatrial node. The sinoatrial region of 63 more hearts of various ages were then studied grossly before and after meticulous removal of the epicardium. A method of sectioning was then devised, and 35 more hearts were then sectioned by this technique and studied histologically.

Histologic examination was thus carried out on a total of 53 formalin-fixed hearts. The muscle fibers of the sinoatrial node, in addition to the collagenous, elastic, reticular, and nerve fibers, were studied by means of hematoxylin and eosin, Weigert's elastic tissue stain counterstained with the Van Gieson stain, phosphotungstic acid hematoxylin, Gomori's trichrome stain, and Hortega's reticulum stain.

GROSS ARCHITECTURE OF SINOATRIAL REGION

The sinoatrial node was originally described by Keith and Flack [4] as lying in the upper part of the sulcus terminalis. This region at birth presents itself in the fixed heart as a brownish gray slightly depressed area, 1 to 3 mm. wide, extending from the recess of the right atrial appendage downward for a varying distance. This area is of slightly whiter or paler texture than the surrounding atrial musculature (Fig. 1). It may be clearly demarcated grossly, or it may be continuous with a white area on the superior surface of the right atrium, immediately adjacent to the hump of the atrial appendage. On the anterior (ventral) wall it may fuse with a white area over the intercaval band. Its two lateral margins may send various offshoots of gray-white tissue over the superior vena cava and the right atrium. In some instances fibers can be seen streaking through this region which are parallel to the longitudinal direction of the sulcus.

With advancing age to maturity, this region becomes whiter in color, blending with the increasing whiteness of the superior vena cava, the interatrial band, the atrium, and the superior atrial wall. Histologically this white opacity is seen to be due to a proliferation of collagenous and elastic fibers in and beneath the epicardium. At about 30 years of age fat becomes identifiable grossly in various locations surrounding the sulcus or in the sulcus. Thus a fat pad appears on

From the Research Laboratories of Mount Sinai Hospital, Miami Beach, Fla. (Dr. Lev), and the Department of Pathology, University of Illinois College of Medicine, Chicago (Dr. Lev and Dr. Watne).

This investigation was supported by a grant from The Heart Association of Greater Miami and by a grant (H-430-C_2) from The National Heart Institute of The National Institutes of Health, United States Public Health Service.

A routine method for the histopathologic study of the sinoatrial node has to date not been devised. The present report deals with such a method. Previous, similar studies have dealt with the atrioventricular node, bundle, and bundle branches.*

* References 1, 2, and 3.

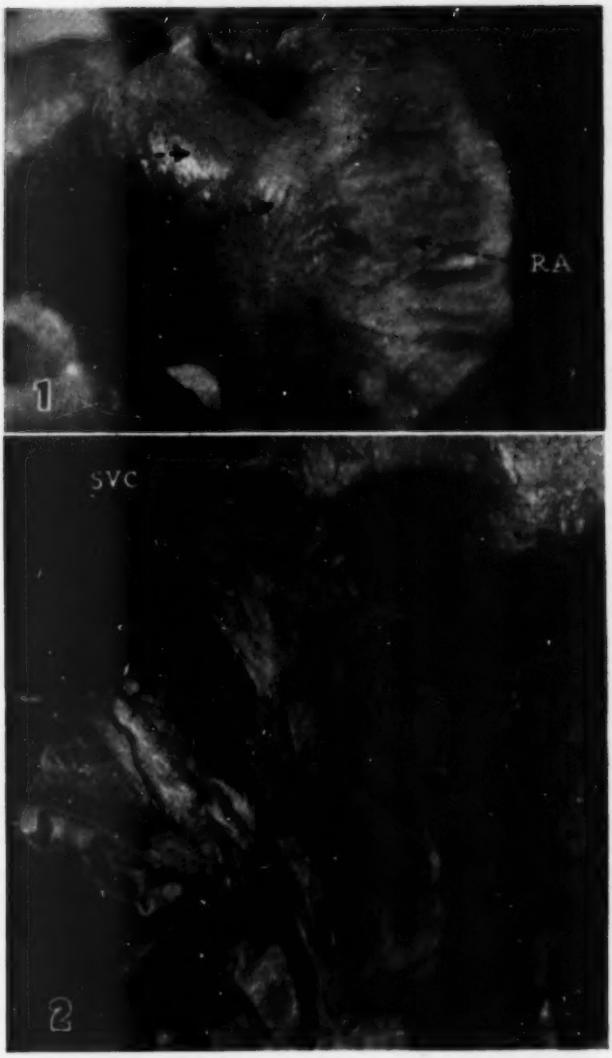


Fig. 1 (Top).—Region of the sinoatrial node in a newborn child. *SVC*, superior vena cava; *ST*, sulcus terminalis; *RA*, right auricle.

Fig. 2 (Bottom).—Region of sinoatrial node with epicardium removed. *SVC*, superior vena cava; *SA*, sinoatrial node; *RA*, right atrium; *R*, recess of right atrium; *IB*, intercaval band.

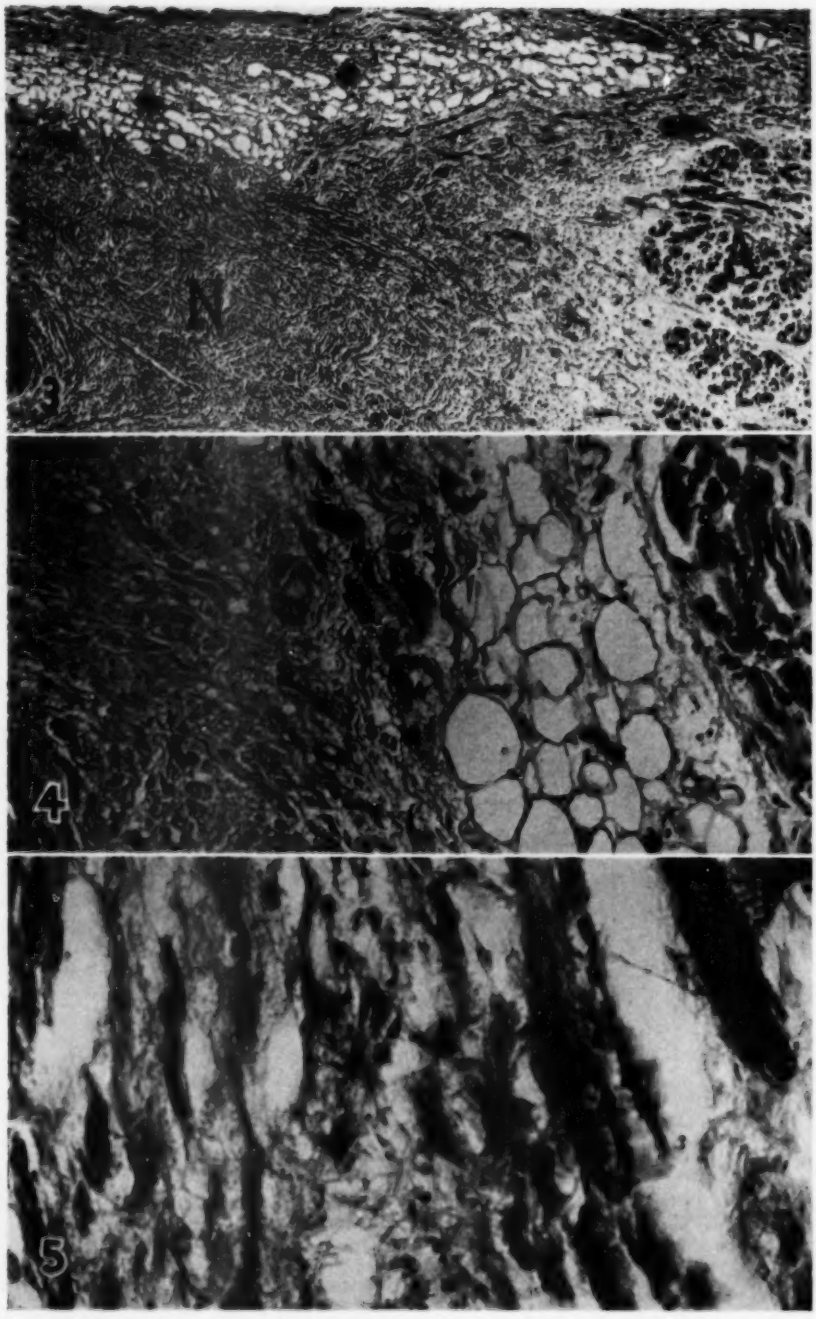


Fig. 3 (Top).—Transverse section through the sinoatrial node in a 40-year-old man. *N*, sinoatrial node; *A*, right atrium. Hematoxylin and eosin; × 57.

Fig. 4 (Middle).—Transverse section through the sinoatrial node in a 65-year-old man. The node is seen to the left of the viewer, with atrial musculature to the right. Hematoxylin and eosin; × 210.

Fig. 5 (Bottom).—Section through sinoatrial node and atrial fibers to show myofibrils and striations. Note the small nodal fibers with relatively scant striations to the left of the viewer, as compared to the atrial fibers to the right. Phosphotungstic acid hematoxylin; × 871.

Fig. 6 (Top).—Transverse section through the sinoatrial node and adjacent atrium of a 40-year-old man. *N*, sinoatrial node; *A*, atrium. Weigert-Van Gieson stain; × 57.

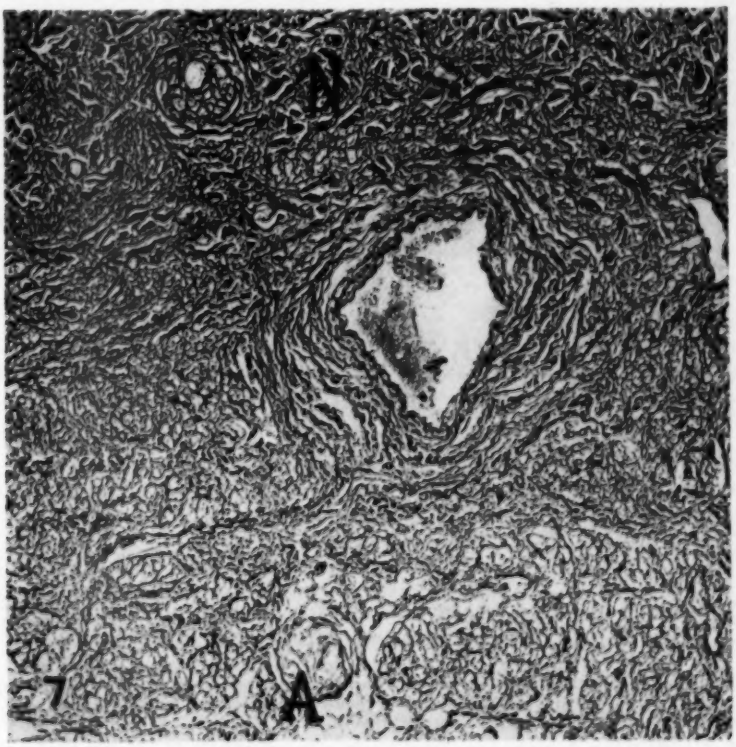


Fig. 7 (Bottom).—Transverse section through the sinoatrial node and adjacent atrium of a 40-year-old man. *N*, sinoatrial node; *A*, atrium. Hortega reticulum stain; × 57.

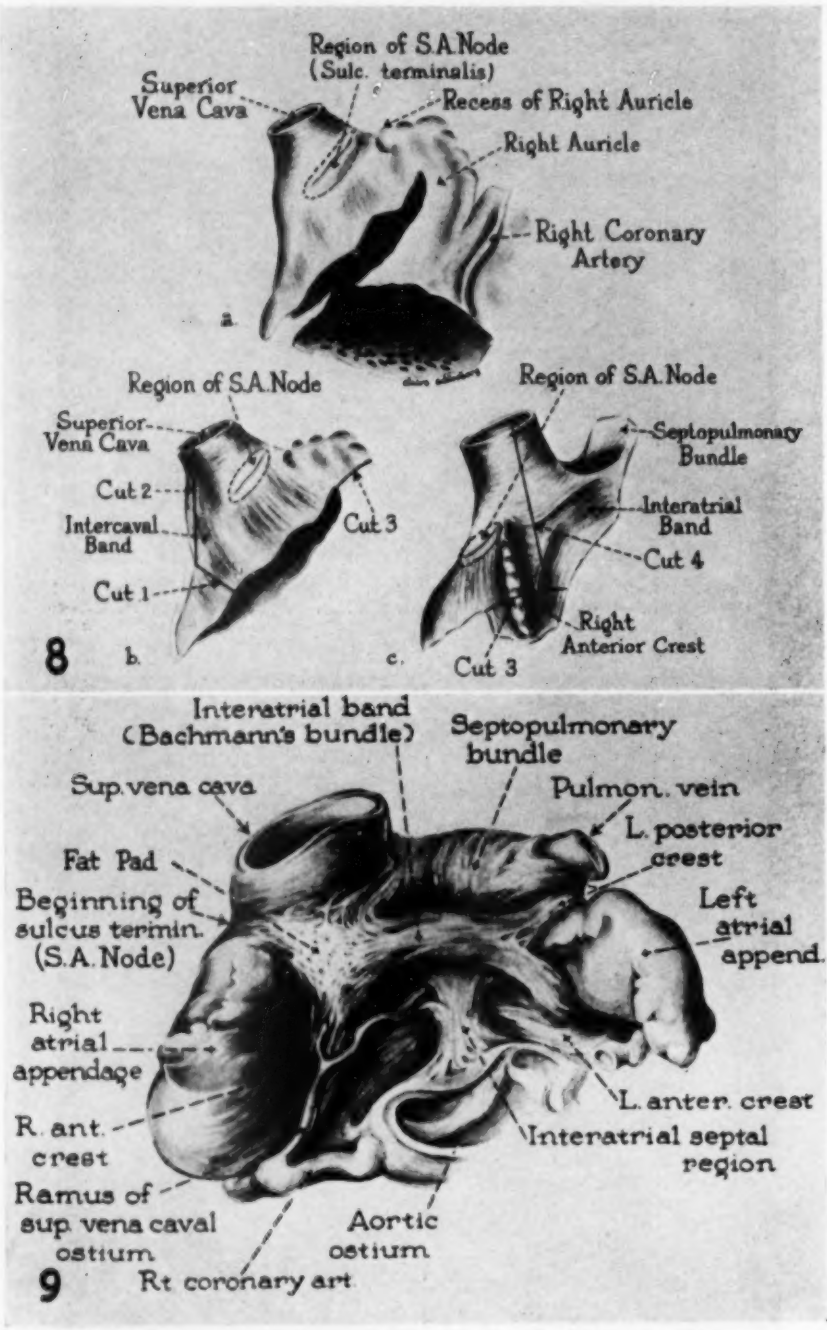


Fig. 8 (Top).—Diagrammatic sketches of the sinoatrial node region, with the method of cutting described in text (*a*, ventral view before removal of the epicardium; *b*, ventral view after removal of the epicardium; *c*, ventrosuperior view).

Fig. 9 (Bottom).—Diagrammatic sketch of the superior surface of the two atria showing Bachmann's bundle.

the superior atrial wall adjacent to the recess, over the intercaval band, or over the superior vena cava. The entire sulcus and its surroundings may be covered with fat. Histologically, fat can be identified in this region as early as 5 years of age. In about 30% of the cases a vessel peers from beneath the epicardium, running in or slightly lateral to the upper part of the sulcus.

Upon removal of the epicardium at birth and in youth, a pale brown fibrillar structure can be seen lying in the upper part of the sulcus terminalis (Fig. 2). Its fibers are parallel to the longitudinal axis of the sulcus. Even at this age it is not always possible to delineate its extent, since it fuses with the white opacities noted above. With advancing age it is still more difficult to demarcate its lateral boundaries. This structure histologically is seen to be the sinoatrial node. Thus, although this node is identifiable in most cases grossly, its exact extent cannot be definitely ascertained by gross dissection. This is due to the fact that the progressively increased amount of collagenous and elastic tissue in its structure fuses with the surrounding collagenous and elastic tissue. This agrees with the opinions of Pace [5] and Taussig.[6]

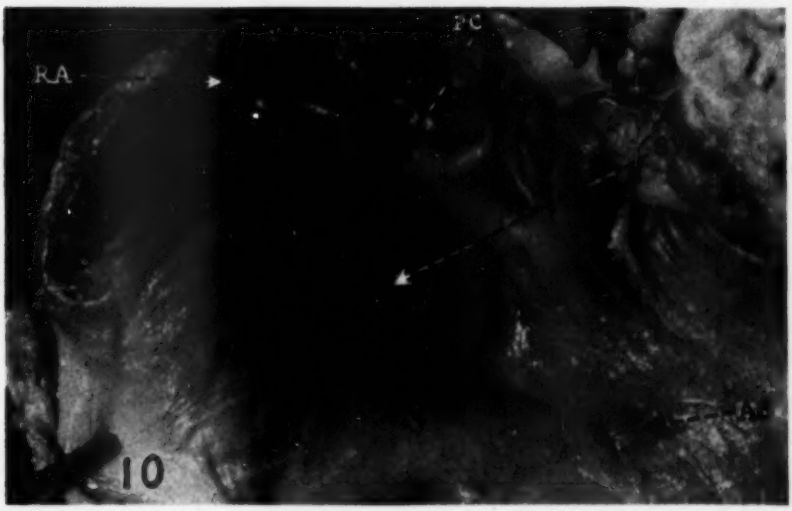


Fig. 10.—View of the superior vena caval orifice, showing the posterior crest. *SVC*, superior vena cava; *PC*, posterior crest; *RA*, right atrium; *AS*, atrial septum.

MICROSCOPIC ARCHITECTURE OF THE SINOATRIAL NODE

Although grossly the sinoatrial node cannot be definitely dissected, histologically the structure is easily identified, even in the lowest magnification (Fig. 3). The structure consists of a plexiform network of slender muscle fibers. These are slenderer than the atrial fibers in all age groups, and the nuclei are of slightly smaller diameter (Fig. 4). The fibers possess a relatively scant number of myofibrils (Fig. 5), with cross striations which are less prominent than those of the atrial myocardium. No distinct intercalated discs are in evidence. These muscle fibers lie in a sea of collagenous and elastic fibers which in all age groups are more copious than those of the atrial myocardium (Fig. 6). The reticular pattern consists of a fine mesh of lightly stained fibers which in all age groups is in contrast to the coarser network of more darkly stained reticular fibers enveloping the atrial myocardium (Fig. 7). Nerve elements are in evidence in the periphery and in the midst of the superior portion (head). The nodal muscle fibers make connections with the musculature of the superior vena cava, the confluence of the right anterior crest and the interatrial band, the intercaval band, and the right atrial appendage. These connections occur without the intervention of Purkinje fibers.

In the adult the node varies from 1.5 to 2.5 cm. in length and from 0.4 to 0.8 cm. in width. In the region of the head its center portion extends from the epicardium almost to the endo-

cardium. Its tail penetrates deeply to approach the endocardium. The node extends from the recess of the right atrial appendage to the region of the intercaval band (Figs. 8 and 9) lying on and lateral to the posterior crest (Fig. 10). The recess of the right atrial appendage is a term we are applying to a small cavity continuous with the main auricular chamber. This cavity overlaps or is contiguous with the uppermost part of the sulcus terminalis. The intercaval band (Wenckebach's bundle) is a muscle which arises from the left and posterior aspect of the superior vena caval junction with the right atrium, curves around to bridge the sulcus terminalis, and then fuses with the atrial musculature. The posterior crest is a thick muscle ridge extending from the atrial septum over the mouth of the superior vena cava to the right anterior wall of the right atrium, where it fuses with the atrial musculature.

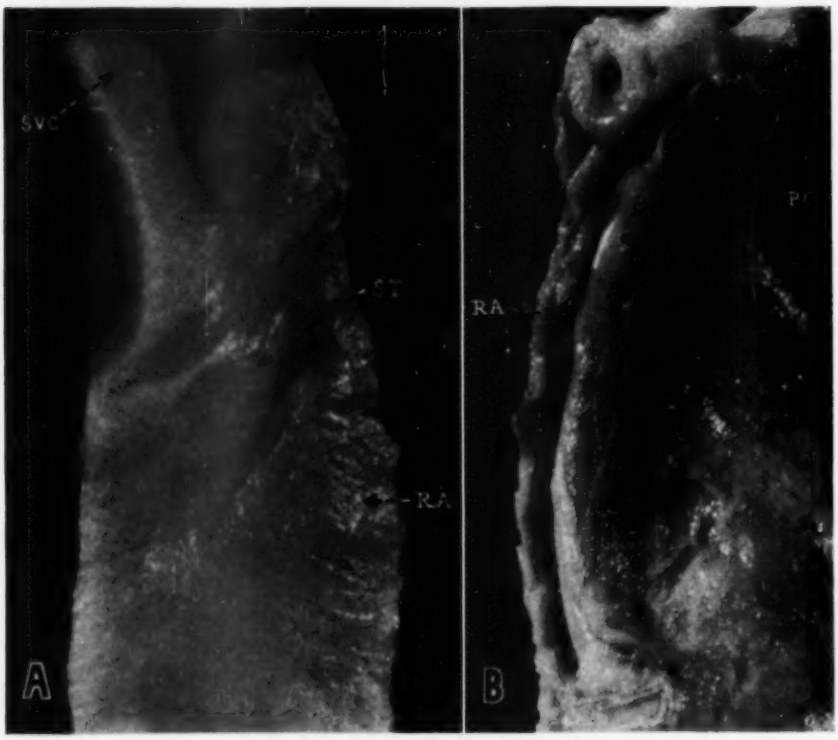


Fig. 11.—Block of tissue removed for sectioning (adult heart). *A*, epicardial view. *SVC*, superior vena cava; *ST*, sulcus terminalis; *RA*, right atrium. *B*, endocardial view. *PC*, posterior crest; *RA*, right atrium.

The above observations confirm and extend the work of Keith and Flack,[4] MacKenzie,[7] Koch,† Burian,‡ Blair and Davies,[10] Davies,[11] Nonidez,[12] and Stotlar and McMahon.[13] They are not in accord with the findings of Tandler,§ as concerns striations; Walmseley,‖ as concerns nodal connections with the atrial musculature; Taussig,[6] as concerns the intervention of Purkinje fibers, and Todd [16] and Glomset and Glomset,[17] as concerns the histologic specificity and identity of the sinoatrial node.

† Koch,[8] cited by Glomset and Glomset.[17]

‡ Burian,[9] cited by Glomset and Glomset.[17]

§ Tandler,[14] cited by Blair and Davies.[10]

‖ Wamseley,[15] cited by Glomset and Glomset.[17]

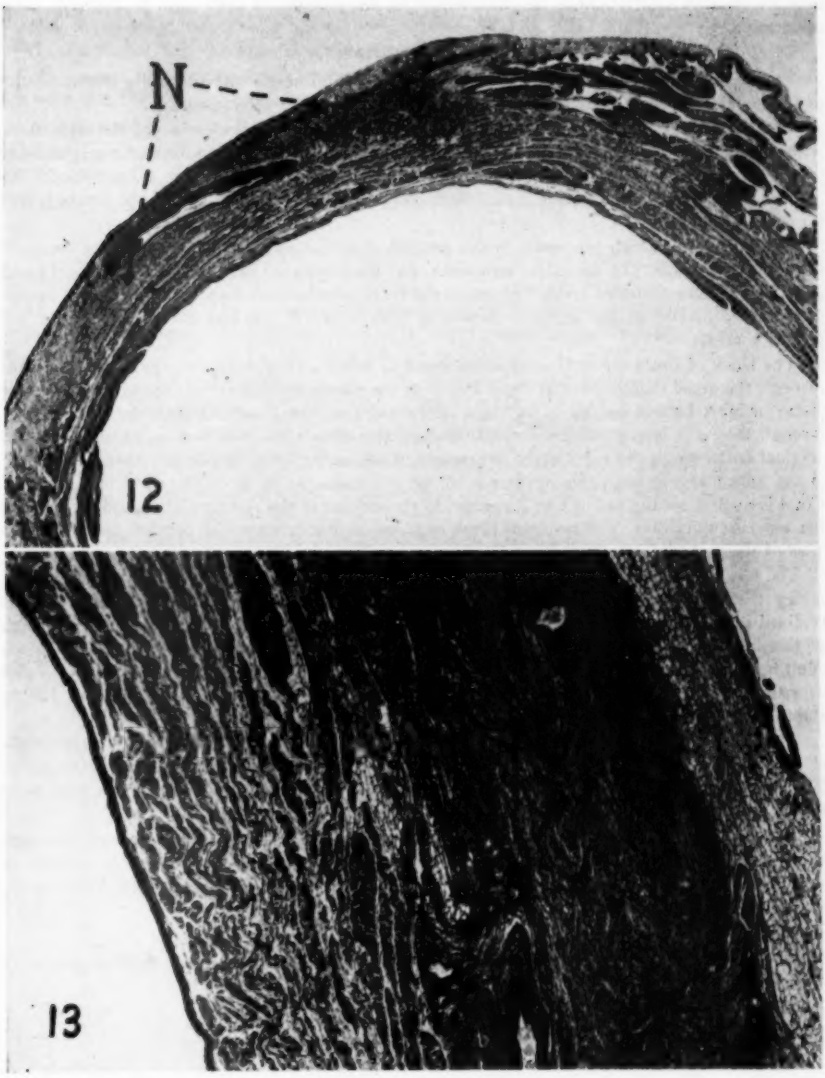


Fig. 12 (Top).—Section obtained by method of cutting described in text. Section shows head and body of node. *N*, sinoatrial node. Hematoxylin and eosin; × 8.

Fig. 13 (Bottom).—Section obtained by method of cutting described in text. Section passes through body of node. *N*, sinoatrial node. Hematoxylin and eosin; × 23.

With advancing age the node grows more slowly than the remainder of the atrial myocardium. There is a progressive increase in elastic and collagenous fibers, an accentuation of the reticular network, and an infiltration of fat. This aspect of the problem is dealt with in a special communication.[18]

METHOD OF SECTIONING

The above studies revealed the exact location and the histologic differentiating characteristics of the node. This enabled us to devise the following method of sectioning.

The heart at autopsy is opened in the usual manner, following the course of the circulation, with one exception. The conventional cut from the inferior to the superior vena cava is changed to a cut passing from the inferior vena cava through the atrial appendage (Oppenheimer[19]). The superior vena caval region is packed with cotton, and the heart is fixed in 4% formaldehyde for 48 to 72 hours.

The landmarks which are useful in our method of sectioning the node are (1) the recess of the atrial appendage, (2) the sulcus terminalis, (3) the interatrial band, (4) the intercaval band, and (5) the right posterior crest. The interatrial band (Bachmann's band [Fig. 12]) is a prominent muscle bundle on the superior surface of both atria. It connects the right anterior and posterior crests.

The block of tissue for sectioning is fashioned as follows (Fig. 8 [*b* and *c*]): Cut 1 is made through the atrial musculature at right angles to the sulcus terminalis, in the region where the posterior crest flattens out, up to the right lateral margin. Cut 2 extends from the end of Cut 1 through the right lateral margin through the superior vena cava. Cut 3 is an extension of the original cut through the right atrial appendage, made at the time of autopsy, over the summit of the appendage through the superior wall for a distance of ¼ to ½ in. (0.64 to 1.27 cm.). Cut 4 is made from the end of Cut 3, parallel to the summit of the right atrial appendage through the superior vena cava. The resultant block of tissue is now trimmed by parallel cuts along the lines of the posterior crest and ¼ to ½ in. lateral to the sulcus terminalis.

The block so obtained (Fig. 11 [*A* and *B*]) is embedded in paraffin, with the posterior crest facing the microtome knife. Serial sections are cut at 6-μ thickness. Every 80th section is retained for study for a total of six sections in the beginning of the block. Thereafter, every 20th section is retained through the remainder of the block. This yields about 50 to 75 sections in the adult heart. The node appears in 15 to 25 of these sections. Sections are alternately stained with hematoxylin and eosin and Weigert's elastic tissue stain counterstained with the Van Gieson stain.

The above method of sectioning presents the sinoatrial node in a longitudinal or longitudinally oblique aspect (Figs. 12 and 13). The node can be recognized by a hand lens on routine stains. The node is especially well seen on elastic-Van Gieson stains because of the copious amount of elastic tissue present.

The method, as presented, is a semiquantitative method of evaluating pathologic findings. Work is in progress to see how much of the pathologic findings is lost in this manner of sectioning, as in the previously reported method of sectioning of the atrioventricular node, bundle, and bundle branches.

CONCLUSION

A semiquantitative method of studying the human sinoatrial node histopathologically is described.

Technical assistance was given by Mr. John T. Thomas.

REFERENCES

1. Lev, M.; Widran, J., and Erickson, E. E.: A Method for the Histopathologic Study of the Atrioventricular Node, Bundle, and Branches, A. M. A. Arch. Path. **52**:73, 1951.

2. Widran, J., and Lev, M.: The Dissection of Atrioventricular Node, Bundle and Branches in Human Heart, Circulation **4**:863, 1951.

3. Erickson, E. E., and Lev, M.: Aging Changes in the Human Atrioventricular Node, Bundle, and Bundle Branches, J. Gerontol. **7**:1, 1952.

4. Keith, A., and Flack, M.: The Form and Nature of the Muscular Connections Between the Primary Divisions of the Vertebrate Heart, J. Anat. & Physiol. **41**:172, 1907.

5. Pace, D.: Dix années de recherches sur le tissu spécifique du cœur, Arch. mal. cœur. **17**:193, 1924.

6. Taussig, H. B.: On the Boundaries of the Sino-Auricular Node and the Atrio-Ventricular Node in the Human Heart, Bull. Johns Hopkins Hosp. **48**:162, 1931.

7. MacKenzie, I.: The Excitatory and Connecting Muscular System of the Heart: A Study in Comparative Anatomy, in Transactions of the 17th International Congress of Medicine, Sec. 1, Anatomy and Embryology, London, 1913, Pt. 1, p. 121.

8. Koch, W.: Welche Bedeutung kommt dem Sinusknoten zu? Med. Klin. **7**:447, 1911.

9. Burian, F.: Zur Histologie des Sinusknotens des menschlichen Herzens, Anat. Anz. **59**:306, 1925.

10. Blair, D. M., and Davies, F.: Observations on the Conducting System of the Heart, J. Anat. **69**:303, 1935.

11. Davies, F.: The Conducting System of the Vertebrate Heart (Hunterian Lecture, Abridged), Brit. Heart J. **4**:66, 1942.

12. Nonidez, J. F.: The Structure and Innervation of the Conductive System of the Heart of the Dog and Rhesus Monkey, as seen with a Silver Impregnation Technique, Am. Heart J. **26**:577, 1943.

13. Stotler, W. A., and Mahon, R. A.: The Innervation and Structure of the Conductive System of the Human Heart, J. Comp. Neurol. **87**:57, 1947.

14. Tandler, J.: Anatomie des Herzens, Jena, Gustav Fischer, 1913.

15. Walmseley, T.: The Heart, London, Longmans, Green & Co., Ltd., 1929, p. 88.

16. Todd, T. W.: The Specialized Systems of the Heart, in Special Cytology: The Form and Functions of the Cell in Health and Disease; A Textbook for Students of Biology and Medicine, edited by E. V. Cowdry, New York, Paul B. Hoeber, Inc., 1928, Vol 2.

17. Glomset, D. J., and Glomset, A. T. A.: A Morphologic Study of the Cardiac Conduction System in Ungulates, Dog, and Man: The Sinoatrial Node, Am. Heart J. **20**:389, 1940.

18. Lev, M.: Aging of the Human Sinoatrial Node, J. Gerontol, to be published.

19. Oppenheimer, B. S.: A Routine Method of Opening the Heart with Conservation of the Bundle of His and the Sino-Auricular Node, J. A. M. A. **59**:937, 1912.

News and Comment

Annual Meetings.—In conjunction with the Annual Meeting of the American Academy of Forensic Sciences, to be held in Chicago, Feb. 25-27, 1954, the Section on Pathology will hold a Medico-Legal Slide Seminar, to be moderated by Dr. Arnold Strauss, Pathologist to the Chief Medical Examiner's Office, Norfolk, Va. This seminar is designed to cover a wide range of problems frequently encountered by forensic pathologists. The sets of slides and the protocols may be obtained by writing to Dr. Geoffrey T. Mann, Chairman, Section on Pathology, American Academy of Forensic Sciences, 404-406 N. 12th St., Richmond 19, Va. Each application must be accompanied by a check for $5 payable to Dr. Mann.

Appointments.—Dr. Gerrit Willem Hendrik Schepers, of Johannesburg, South Africa, has been named Director of the Saranac Laboratory of the Trudeau-Saranac Institute, Trudeau, N. Y. He is expected to assume his new duties in the late spring of 1954.

Under Dr. Schepers' direction it is expected that the work of the Saranac Laboratory conducted by previous directors, Drs. Edward R. Baldwin, Leroy U. Gardner, and Arthur J. Vorwald, will be continued and expanded.

Histochemical Society.—The Histochemical Society will meet at Atlantic City, N. J., April 16-17, 1954, after the session of the American Society for Experimental Pathology. A symposium on "Basophil Components of Cytoplasm" will be held Friday afternoon, April 16.

Paragon Tray Drawer Cabinet

Compact

U. S. Pat. No. 2,202,047
C101—Tray Drawer Cabinet for 3 x 1 Micro Slides
Capacity 4500—18¾ x 15¾ x 4¾

Low Cost

FOR FILING
MICROSCOPIC SLIDES 3 x 1"
KODACHROME TRANSPARENCIES
2 x 2" SLIDES
LANTERN SLIDES
(up to 3¼ x 4¼)
PETROGRAPHIC SLIDES

When you purchase a
PARAGON TRAY DRAWER CABINET YOU PURCHASE FILING SPACE ONLY NO WASTE SPACE-EVERY INCH USED

All Paragon Tray Drawer Cabinets are manufactured in standard sizes so that any number of sections may be interlocked to form one cabinet to accommodate any number of varied slides. The dimensions of the different cabinets are the same as to length and width, varying only in height. The cabinet formed by interlocking may be 18¾ x 15¾; 18¾ x 11 or 18¾ x 5 or it may be a pyramid with the sections varying in width.

C221—Capacity 1500 Slides—18¾ x 11 x 3¾
For Filing KODACHROME TRANSPARENCIES and 2 x 2" SLIDES

SPECIFICATIONS: All Paragon Tray Drawer Cabinets are made of reinforced steel construction, olive green finish. Interlocking device enables several units to be joined into one. Each sectional unit contains removable drawers with hand grip in front and rear. Interlocking steel base obtainable whenever required. **Constructed according to rigid specifications—not merely adapted.**

Address your orders and inquiries to Dept. P.
Manufactured Exclusively by

PARAGON C. & C. CO., Inc. • 2540 Belmont Ave., New York 58, N. Y.

No. 60-660

$10.00

Request bulletin 150-E describing all cabinets

Eberbach Expandable Unit System Cabinets Hold 500 Slides Flat

The popular Eberbach Unit System filing cabinets hold 500 3" x 1" slides flat in separate compartments. The filing cabinets are built with flat sides and top to facilitate "stacking" as the need for additional filing capacity arises. The 25 numbered aluminum drawers each hold 20 slides in separate compartments. The durably finished oak cabinets have a disappearing door and measure 8½" x 10" x 12" deep.

Eberbach CORPORATION
ANN ARBOR, MICH.

PLASTIC COVERSLIPS
patented *

EXCELLENT for ROUTINE BLOOD SMEARS, etc.

- ¼ THE PRICE OF GLASS
- LAY FLAT
- UNBREAKABLE
- WILL NOT CUT FINGERS

ALL SIZES AVAILABLE . .
. . . ROUND or RECTANGLES

Up to and including 18 mm—$2.75—M
22 mm round or square up to 22 x 26—$3.00—M
18 and 22 mm only, in hundreds boxes: 50c M—more
22 x 30 to 24 x 60 mm—$5.05—M

MINIMUM ORDER 10,000

ROBERT BUSSE CO. INC.

111 BROAD ST., N. Y. 4, N. Y.
OR YOUR DEALER WHO ALSO SUPPLIES SMALLER QUANTITIES
*Patented by Robert Busse

▶ *available on request . . .*

"THE LATEST INFORMATION ON FREEZE-DRYING"

Our research department has prepared a bulletin *"Freeze-Drying of Tissues—Present Status of Technology"* which gives information on how to make freeze-drying a practical everyday procedure in your laboratory. In addition to its vital role in the research laboratory this important new technique is becoming a necessary part of college and university courses in histology. This illustrated bulletin contains answers to many questions that have been asked of us concerning freeze-drying equipment and techniques. The scientific literature in this field has lagged behind our progress in instrument development. For this reason, quantitative data has been prepared showing the efficiency of our simplified design. Included are complete details on this inexpensive, easy to use equipment.

Histological Freeze-Drying Unit, Model FD-11. Complete with accessories . . . $575 f.o.b., Boston, Mass.

SCIENTIFIC SPECIALTIES CORP.

Manufacturers of biological, medical, electronic, and optical equipment, also research, development, custom designed scientific equipment.

Write: Snow and Union Streets Boston 35, Mass.

FISHER *Tissuemat*

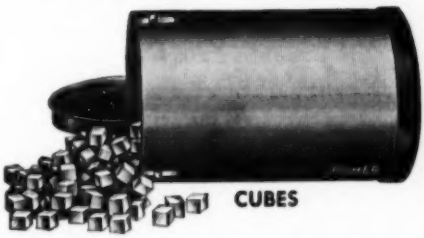
THE SUPERIOR EMBEDDING COMPOUND

Tissuemat is widely used by leading laboratories for impregnating and embedding biological specimens for microscopic examination.

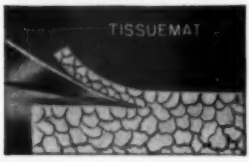
Fisher Tissuemat is superior because:

1. It will not crack or crumble during slicing
2. Forms ribbons readily
3. Coheres during slicing
4. Convenient and clean to handle

FLAKES



CUBES



The larger cell structure of Tissuemat makes it superior to paraffin because it will not crack or crumble when cut on the Microtome. There is no cell shrinkage or cell distortion during impregnation or cutting.

Tissuemat Flakes are packed in one-pound cartons with moistureproof liners and in ten-pound fiber containers. When ordering, specify melting point range.

1-lb.....$.70 10-lbs.....$6.50

Tissuemat Cubes are about one inch thick, convenient for quick dispensing. The containers are fiber with metal ends.

25-lbs....$13.50 50-lbs....$25.00

Melting point ranges available

A. 50-52°C. (122-125°F.) C. 54-56°C. (130-133°F.)
B. 52-54°C. (125-130°F.) D. 56-58°C. (133-137°F.)
E. 60-62°C. (140-143°F.)

MICROTOMES are available in several models to meet various requirements. Special Microtome knife sharpener also available. Write for complete details.

***FREE*—one pound sample from us or ask your dealer. Specify melting point.**

FISHER SCIENTIFIC

PITTSBURGH (19) 717 Forbes St. | NEW YORK (14) 635 Greenwich St. | ST. LOUIS (18) 2850 S. Jefferson Ave. | WASHINGTON 7722 Woodbury Dr., Silver Spring, Md. | MONTREAL, P.Q. 904 St. James | TORONTO (8), P.O. 245 Carlaw Ave.

America's Largest Manufacturer-Distributor of Laboratory Appliances and Reagent Chemicals

what used to take *days*

To fully appreciate the significance of Autotechnicon's automatic processing, one needs only recall the tissue-processing practices in the pathologic laboratory prior to its introduction. Ten, twenty (or many more) containers of reagents spread along a table, over which a technician wearily presided, hour after hour, shifting tissues from one solution to another. The slow pace of the procedure was matched only by the uncertainty of the result.

By contrast, brisk overnight service is routine in today's Autotechnicon-equipped laboratory. Finished tissues are ready within short hours of surgery. Processing is completely mechanized . . . requires no human intervention or supervision. Results are predictably uniform; every step at every stage is strictly governed, timed to hairbreadth precision.

is now done in *hours* by the

Autotechnicon®

every phase of tissue processing *automatically* . . .
fixation, washing, dehydration, infiltration, staining

THE TECHNICON COMPANY, 215 East 149 Street, New York 51, N. Y.